

THE AMERICAN JOURNAL
OF PATHOLOGY

THE AMERICAN JOURNAL OF PATHOLOGY

*Official Publication of
The American Association of Pathologists and Bacteriologists*

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VOLUME X

1934

BOSTON
MASSACHUSETTS
U. S. A.

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PRINTED AT THE HARVARD UNIVERSITY PRESS
CAMBRIDGE, MASS., U. S. A.

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VOLUME X

JANUARY, 1934

NUMBER 1

CARCINOMA OF THE TUBES AND OVARIES SECONDARY TO CARCINOMA OF THE BODY OF THE UTERUS *

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It is the purpose of this paper to present the results of the study of a small series of judged primary carcinoma of the body of the uterus associated with judged secondary carcinoma, either in the tube, the ovary, or in both organs. An attempt was made to determine the pathogenesis of the secondary carcinomas, with special reference to the justification of the theory that cancer cells escaping from the uterine tumor may be transported through the lumen of the tube and may become implanted in the tubal mucosa and on the surface of the ovaries and peritoneum.

It is a reasonable assumption that carcinoma in any two or all three of these organs may arise in various ways. The close anatomical relations between the uterus, tubes and ovaries, often made more intimate by an advanced carcinoma in one, may readily permit the continuous extension of the growth from one organ to another through normal preëxisting structures, or by the affected organ becoming adherent to one of the others. The common lymphatic and venous circulation of the three organs offers channels through which carcinoma may extend from one organ to another by continuous permeation; or emboli of cancer cells escaping into the main vessels may be diverted to the tubes or ovaries in channels where the stream is often sluggish, especially if there is the least temporary or permanent obstruction beyond. The lumina of the tubes afford avenues not only for the transportation of sperm and ova, but also for bacteria and cancer cells.

* Received for publication June 26, 1933.

The common ancestral origin of the germinal epithelium of the ovary, from which many of the ovarian carcinomas directly or indirectly arise, and that of the tubal and uterine epithelium offers a foundation for the simultaneous development of carcinoma in any two or all three of these organs, namely, carcinoma of multicentric origin in closely allied organs. Since primary carcinoma occurs in all three organs it is natural to assume that, in some instances of this disease in two or even in all three, the growths in the different situations might be independent of each other. Nevertheless, carcinomas of multicentric origin in the same or different organs are of infrequent occurrence. When multiple carcinomas of the same histological structure are present in an individual, it usually indicates a primary growth in one situation with secondary tumors in the others.

It is generally accepted by both clinicians and pathologists that carcinoma usually spreads by continuous extension and by metastases through the lymphatics. Therefore it is quite natural to attempt to explain the presence of this growth in any two or all three of these organs as having arisen from a primary neoplasm in one with the development of secondary tumors in the other situations, as a result of continuous extension of the primary tumor or lymphatic metastasis.

Observation of conditions found at operation, together with a careful study of the specimen removed, should enable one, definitely, to determine whether or not the tumors in the different organs are continuous. On the other hand, the evidence of the pathogenesis of any seemingly metastatic growth is, at best, merely circumstantial.

When the growth in the different organs is in contact with the epithelium of these organs the multicentric theory is alluring if continuous extension can be excluded and evidence of metastasis through well recognized channels is not present.

THE STATUS OF THE TRANSTUBAL IMPLANTATION OF CARCINOMA

Reichel,¹ in the year 1888, reported two cases of ovarian carcinoma, each associated with a small carcinoma of the uterine mucosa. In the discussion of the etiology of the uterine carcinomas in these two cases he introduced the theory that they arose from the implantation of cancer cells, which had escaped from the ovarian carcinoma

through the lumina of the tubes, on the surface of the uterine mucosa. He compared their pathogenesis with the phenomenon of the passage of the fertilized ovum through the tube and its implantation in the uterine mucosa.

Sitzenfrey,² in the year 1908, reported a case of carcinoma of the uterus in which free particles of the uterine growth were found in the lumen of one of the tubes without the presence of carcinoma of the tubal mucosa. He believed that secondary tubal carcinoma may arise from this source and that with a further migration of these particles implantations of this growth on the surfaces of the ovary and peritoneum may arise.

In 1922 Schiller³ reported a similar case. He agreed with Sitzenfrey as to the possible etiology of secondary tubal, ovarian and peritoneal carcinoma from this source.

In 1924 I published a paper⁴ entitled: "Benign and Malignant Endometrial Implants in the Peritoneal Cavity and their Relation to Certain Ovarian Tumors." It was shown, in that paper, that fragments of malignant endometrial tumors, at times, escaped into the lumina of the tubes. Evidence was presented indicating that implantations of these particles sometimes occurred in tubal mucosa, and on the surfaces of the ovaries and peritoneum. Two cases reported in the previous paper will again be presented in the present one, with additional photomicrographs showing the secondary tubal and ovarian tumors. Later in the same year Norris and Vogt⁵ reported an instance of an early metastasis of carcinoma on the surface of the ovary from carcinoma of the uterus. They believed the ovarian carcinoma arose from the implantation of cancer cells escaping through the lumen of the tube during a diagnostic curettage six weeks before.

In 1927 Novak,⁶ in a paper entitled "Ovarian Metastasis with Cancer of the Uterine Body: Is Transtubal Implantation an Important Factor?" presents an excellent summary of the evidence that the lymphatics are the chief channels for metastases of uterine carcinoma to the ovary and *vice versa*. He most emphatically endorses these channels as avenues for the dissemination of carcinoma from the uterus to the ovary, and just as emphatically denounces the transtubal route. He states: "The cases reported by Sampson of supposed implantation cancer of the ovary are far more logically explained as due to lymphatic dissemination."

In 1930 Robinson,⁷ in an interesting paper on primary and secondary ovarian carcinoma, denounces the implantation theory as applied to the origin of metastatic ovarian carcinoma secondary to the intestinal tract, and warmly supports the lymphatic route. He states: "The soundest of all theories formulated about the manner and method of cancer metastasization is the one of the lymph and vessel routes, and this theory is easily and frequently verified. In fact, some authorities admit of no other possibility." Robinson believes that the simultaneous appearance of malignant papillary tumors, within ovarian cysts and upon the surface of the ovaries, and similar growths of the uterine and tubal mucosa, as well as the serosa covering these organs, is due to the same biological factors influencing variously located and metamorphosed epithelial centers of a common genetic source.

In 1932 Offutt⁸ published the results of an analysis of a series of cases from the Mayo Clinic in which carcinoma was present in both the ovaries and uterus. There were 520 cases of carcinoma of the body of the uterus and 616 of papillary cystadenocarcinoma of the ovaries, in which operation had been performed in the clinic from 1913 to 1930. In fifty-three of these cases carcinoma was present in both organs. In five of these cases the uterus was judged to be the site of the primary neoplasm. Carcinoma was present in a Fallopian tube in three of these. In sixteen, one or both ovaries were judged to be the site of the primary growth, in eight of which one or both tubes also contained carcinoma. In thirty-one cases the situation of the primary growth could not be determined, in four of these carcinoma was present in one tube. Offutt discusses the various means by which this growth may extend from the uterus to the ovary and *vice versa*. She emphasizes the importance of the transtubal route in the group of cases reported by her. In several instances cancer cells were found in the lumen of the tube of which the blood vessels and lymph channels were apparently normal.

This brief reference to some of the literature dealing with the transtubal migration of cancer cells and the origin of secondary carcinoma of the uterus, tubes and ovaries from this source must impress one, not only with the difficulty of properly evaluating this channel, but also the natural difference of opinion that must arise as to its importance, and as to whether metastases ever occur in this way.

I heartily agree with the majority of pathologists who believe that carcinoma is usually disseminated from its primary focus through the lymphatics. However, there is one striking exception to this rule and that occurs in certain types of ovarian carcinoma where implantations on the peritoneum arise from the growth of cells escaping from the primary tumor into the peritoneal cavity. Even in these cases the lymphatics also may be invaded, not only by the primary tumor, but also by secondary tumors on the peritoneum which may have arisen from the primary one by implantation. We must not lose sight of the fact that a secondary tumor may possess the same or even greater potentialities of invasion and dissemination than the growth from which it arose by direct extension or metastasis.

It is generally accepted by both clinicians and pathologists that, as compared with carcinoma of the uterine cervix, metastases to the retroperitoneal lymph nodes rarely occur in carcinoma of the body of the uterus.

It is not my purpose to belittle the importance of the lymphatics or veins as avenues for the dissemination of carcinoma of the body of the uterus, or to minimize the incidence of carcinoma of multicentric origin, or over-emphasize the part played by the lumina of the tubes in conveying cancer cells from the uterine tumor to that organ and thence to the surface of the ovary and peritoneum. However, I desire to present what was found in this series of cases, to give my own reactions and gladly leave others to draw their conclusions, not only from my observations but, of greater importance, from the study of material from other cases similar to those which I am to report.

The closest available analogy we have to the implantation of cancer cells of uterine origin on the surface of the ovary and peritoneum is the implantation of cancer cells of ovarian origin on the peritoneum, since genetically the peritoneal mesothelium, the surface epithelium of the ovary and the uterine epithelium have a common ancestor. The results of a previous study⁹ of implantation peritoneal carcinomatosis of ovarian origin demonstrated that it arises from the repair of injuries to the peritoneum caused by cancer cells that have escaped into the peritoneal cavity and lodged on the surface of its serous membrane, together with the continued growth of these cells in this situation. The various stages in this repair, as well as the laws governing the same, are similar to those encountered in the repair of tissues injured by foreign bodies and in the taking of

skin grafts — namely, the healing of wounds. The histological structure of these implants varies with the reaction of the peritoneal tissues before and after the fixation of the cancer cells and with the activity of the latter. As a result carcinoma becomes embedded in the peritoneal scar, encapsulated on its surface, enmeshed in adhesions, or like a surgical skin graft grows on the surface of the peritoneum without encapsulation.

The principles governing the implantation of uterine carcinoma in the mucosa of the tubes should be the same as those governing the taking of a small skin graft, bearing in mind that the cancer graft is derived from epithelium having the same ancestor as the tubal epithelium. The cancer cells must be viable, there must be a break in the epithelial lining of the tube already present or possibly created by the carcinoma, and the graft must be held in place or at least not disturbed until it becomes firmly attached. It is conceivable that such conditions would be encountered in the tube more often than in the intestines or urinary tract, including the bladder. It is also conceivable that cancer cells of uterine origin once grafted in tubal mucosa might bear the same relation to the normal tubal epithelium about it as carcinoma primarily arising from that epithelium. If true, it would be impossible to determine from its structure whether a given tubal carcinoma arose from grafted cancer cells or from a differentiation of tubal epithelium.

MATERIAL AND METHODS OF STUDY

From 1921 to 1932 inclusive, 183 patients with carcinoma of the body of the uterus have been operated upon in the gynecological service of the Albany Hospital. The material from nineteen of these cases was chosen for the present paper and also from one additional case operated upon in 1933. Thirteen of the twenty cases included all instances of carcinoma of the body of the uterus with a similar growth in the tube, ovary or both organs, in which the growth in the uterus was judged to be the primary one.

The criteria upon which this opinion was formed was not only the size and apparent age of the uterine tumor and its histological structure (corresponding with that of uterine carcinoma without carcinoma in the tubes and ovaries), but also the relatively smaller size and apparently younger age of the associated tumors in the other organs. In addition, evidence must be present indicating that carcinoma actually has or may have reached these organs from the uterine growth. I realize that any or even all of these criteria may not be sufficient in a given case for a positive diagnosis of the site of the primary neoplasm. A secondary tumor may be larger and more invasive than its parent and therefore apparently older; and primary carcinoma of the tube and ovary may histologically be

indistinguishable from carcinoma arising in the endometrium. Also, the evidence of the pathogenesis of a metastatic tumor, no matter how convincing, is at best circumstantial and even may be misleading.

The cases of obvious primary carcinoma of the tubes and of large or extensive ovarian carcinomas were not included, even though there was associated with the latter an extensive carcinoma of the uterus. In some of the latter group the primary growth might well have been in the uterus. The relation between carcinomas in these organs is much more difficult to determine when the tumors in both situations are either of the same size or advanced.

The findings in the remaining seven cases of carcinoma of the body of the uterus were chosen on account of their bearing on the pathogenesis of the conditions present in the other cases.

The study of this problem begins in the operating room. On exposing the pelvic contents a careful examination is made to determine whether or not metastases are present. The ovaries and tubes, and especially the fimbriae of the latter, are inspected for any abnormalities suggesting carcinoma in these structures. If present the tubes and ovaries are first removed and immediately placed in formalin. This enables one to obtain material which otherwise might be traumatized during or after the operative removal of the uterus and likewise minimizes the danger of disseminating cancer cells from them into the operative field. When the tubes and ovaries appear normal each tube is ligated just back of the fimbriae to prevent any carcinoma possibly present in the lumen of the tube, or later forced into it by operative manipulation, from escaping through the distal end of the tube. The ovarian and uterine vessels are either doubly ligated and severed between the ligatures, or else ligated and clamped proximal to the ligature before cutting them. This is done to prevent any cancer emboli possibly present in the lumina of the lymphatics and veins from escaping into the field of operation. After freeing the cervix and upper portion of the vagina a right-angle clamp is placed across the vagina below the cervix and a vaginal douche is given before severing the vagina to prevent further soiling of the field of operation.

All specimens are examined before they leave the operating room. Should they present unusual features sketches are made and these are carefully labelled. Specimens not presenting unusual features are sent, as such, to the general pathological laboratory, where they are described and tissue is removed from the uterus, tubes and ovaries, fixed in Zenker's fluid, embedded in paraffin, and cut and stained as a routine procedure.

The routine examination of pathological material is of the greatest value for records and diagnostic purposes, but often is not sufficient for the intensive study of any special problem. Through the courtesy of Dr. Victor C. Jacobsen I have been permitted to retain portions of specimens in which I have been especially interested, saving for him sufficient material for diagnosis and laboratory records.

I employ formalin for fixing the tissue and block it myself. The blocks (often large) are embedded in celloidin rather than paraffin, as it causes less shrinkage and fewer artifacts after formalin fixation. One can then study the surface of the block as the sections are cut. My technician is instructed how the blocks are to be mounted and cut, and, most important, what I wish to ascertain in each block. Much may be learned by a well trained technician while watching the surface of the block as the sections are cut. From time to time, while cutting the block, sections are stained to make sure of the conditions present. If the technician is in doubt the block is not cut further until I have had an opportunity to study the stained sections. All sections are saved until the entire block has been cut and

sections studied. In many situations serial sections are made. This has proved to be of the greatest importance in many instances. Often I have wished for serial sections after the block has been cut. Since two sections cannot be examined under the microscope by one person at the same time, photomicrographs are used extensively by me in the study of these problems. I find them of great assistance in the comparative study of the findings in different sections.

In each instance the methods employed in the study of this material were initiated by the interest aroused by conditions observed at operation or in the inspection of the fresh specimen after its removal. This led to the intensive study of some portions of the specimen and a relatively casual examination of others. As a result there is a lack of uniformity in the manner and thoroughness of the study of all the specimens, and even of all parts of those more or less intensively studied. For these reasons these studies are of little or no statistical value.

CASE REPORTS

CASE 1. Advanced adenocarcinoma of the body of the uterus, which had extended through the posterior uterine wall with resulting invasion of the omentum and the peritoneum of the mesosigmoid which had become adherent over this area; metastases to the subperitoneal tissues of the left uterine cornu with subsequent extension through the overlying peritoneum.

Albany Hospital No. 8029-30. The patient, aged 56 years, complained of uterine bleeding of 7 months duration. A diagnostic curettage was done at the Albany Hospital Nov. 6, 1930. Because of difficulty in persuading the patient to have a major operation, the latter was not done until Nov. 12, 1930. At that operation the omentum and mesosigmoid were found to be adherent to the fundus and posterior surface of the uterus. A small amount of blood-tinged fluid was present in the posterior cul-de-sac. Some of this was removed for microscopic examination of its cellular contents. No gross evidence of metastases was detected. The portion of the omentum adherent to the uterus was excised and the mesosigmoid was freed from the uterus by removing the portion of its peritoneum, which was adherent to the latter. The entire uterus, both tubes, ovaries and appendix were removed. The patient made a satisfactory convalescence but died in a year's time from carcinoma.

A study of the specimen removed (see Figs. 1 to 5 inclusive, with their legends) demonstrated the following: (a) the variations in histological type that different portions of the same cancer may present; (b) the penetration of the uterine wall by the tumor and subsequent invasion of structures becoming adherent to it over this area, the possibility that carcinoma, which has thus penetrated the uterine wall, may escape into the peritoneal cavity and become implanted on the surface of the peritoneum; (c) the presence of subperitoneal metastases of the uterus with cancer cells in the nearby lymph vessels,

the extension of carcinoma in these metastases to the surface of the peritoneum and its possible subsequent escape into the peritoneal cavity; (d) the presence of clumps of possible cancer cells in peritoneal fluid which may have escaped into the peritoneal cavity through the sources already described or through the patent tubes during the curettage six days before the major operation. Carcinoma was not found in the tubes and ovaries.

CASE 2. Advanced adenocarcinoma of the body of the uterus with extension through the posterior uterine wall and subsequent adhesions between it and the sigmoid and terminal loop of the ileum; carcinoma of the left ovary from continuous extension of the uterine growth; subperitoneal metastases of the posterior uterine wall and left tube, and peritoneal carcinomatosis limited to the posterior cul-de-sac.

Albany Hospital No. 8781-31. The patient, aged 72 years, complained of pain and uterine bleeding of 3 months duration. A preliminary diagnostic curettage was not done. At operation Nov. 28, 1931, the uterus was found to be enlarged, due to multiple small myomata, and was slightly adherent to the sigmoid and terminal loop of the ileum. Metastases to the retroperitoneal lymph nodes were not detected. The entire uterus and both tubes and ovaries were removed. In doing so it was noted that peritoneal carcinomatosis was present in the posterior cul-de-sac. The patient made a satisfactory convalescence and felt better for several months after the operation, but died in a little over a year's time.

A study of the specimen removed (see Figs. 6 to 17 inclusive, with their legends) demonstrated: (a) the variations in histological type in the same and different portions of the cancer; (b) the diffuse extension of the carcinoma through the uterine wall, and especially through that of the left cornu; (c) the invasion of the left ovary by direct extension through the left utero-ovarian ligament; (d) subperitoneal metastases of the uterus and left tube, and also a submucosal metastasis of the fimbriae of the left tube. The most interesting and important finding was the extension of the carcinoma through the uterine wall to the surface of the peritoneum, with the development of local peritoneal carcinomatosis from the judged implantation of cancer cells thus escaping into the peritoneal cavity (see Figs. 14, 16 and 17). Since implantation carcinomatosis arose near the site of the penetration of the carcinoma into the peritoneal cavity, a similar phenomenon could well account

for the peritoneal carcinomatosis at a little greater distance, namely, in the posterior cul-de-sac. Carcinoma was not found in the right tube and ovary.

CASE 3. Advanced squamous cell carcinoma of the body of the uterus secondary to that of the cervix, with direct extension to the surrounding structures, metastases to the pelvic lymph nodes and secondary carcinoma of the right tube and ovary, apparently due to continuous lymphatic permeation.

Albany Hospital Nos. 6607-30 and 4727-31. The patient, aged 64 years, was first seen by me Sept. 10, 1930. She had had radium treatment for carcinoma of the cervix in a hospital in another city 6 months before. The cervix was replaced by a necrotic ulcerative mass. The uterus was fixed in the pelvis. The disease was so far advanced that the value of further radium treatment seemed questionable. Nevertheless it was employed. At first the palliative relief was quite marked. The patient was readmitted June 16, 1931, for uncontrollable uterine bleeding. An exploratory abdominal incision was made under spinal anesthesia June 30, 1931, with the plan of ligating the blood supply to the uterus and thus stopping the bleeding. The uterus was about three times its normal size and fixed in the pelvis. One could easily follow the white, distended, sub-peritoneal lymphatics of the right uterine cornu out between the layers of the broad ligament, where they were joined by similarly dilated lymphatics of the right tube and ovary. These lymphatics were evidently filled with carcinoma as with a white injection mass. The retroperitoneal tissues of both sides of the pelvic wall were so infiltrated with the growth that it was impossible to gain access to either the anterior branches of the internal iliac arteries or their uterine branches. The ovarian vessels were enlarged, thus suggesting that the uterus was receiving the greater portion of its blood supply from these sources. The right tube and ovary were removed and the left ovarian vessels and both round ligaments were ligated. The operation did not disturb the patient. The bleeding ceased and never returned. The patient died in June, 1932.

A study of the right tube and ovary (see Figs. 18 and 19, with their legends) demonstrates the location and type of carcinoma of the tube and ovary that may arise from the continuous permeation of the lymphatics by uterine carcinoma.

CASE 4. Advanced adenocarcinoma of the body of the uterus with secondary carcinoma of both tubes, ovaries, round ligaments and peritoneum of the cul-de-sac about the uterosacral ligaments. The secondary growths apparently arose mainly from lymphatic permeation and possibly in some instances from embolic metastases.

Albany Hospital No. 5634-28. The patient, aged 61 years, complained of uterine bleeding of over a year's duration. A preliminary curettage was done, followed by an exploratory incision Sept. 13, 1928. The uterus was enlarged and

fixed in the pelvis, due to extension of the growth through the lymphatics, especially those about the round and uterosacral ligaments with resulting invasion of the peritoneum about the ligaments. Both tubes were enlarged and their walls firm. The ovaries were of normal size. No attempt was made to remove the uterus. The right tube, ovary and left tube were removed and the left ovarian vessels ligated. The patient made a satisfactory convalescence, but died later.

A study of the right tube and ovary (see Figs. 20 to 26 inclusive) demonstrated the permeation of the lymphatics by carcinoma and the origin and type of carcinoma of the tube and ovary secondary to that of the uterus from lymphatic permeation and metastases. We were able to demonstrate, by serial sections, that all the growth in the dilated lymph vessels was not in the form of continuous threads or strands, but that some of it consisted of clumps of cancer cells, namely emboli, floating about in the lumina of the dilated lymphatics. Furthermore, we were able to show that these emboli at times became attached to the lining of these vessels, forming implantations of both the grafted and foreign body (encapsulated) type (Figs. 21 and 23), similar to the metastatic peritoneal tumors frequently found in peritoneal carcinomatosis of ovarian origin.

CASE 5. Advanced adenocarcinoma of the uterus, which had been treated with radium. Two months after the biopsy and radium treatment a papillary metastasis was found at the junction of the vagina and vulva in the scar of a wound of the hymen created at the time of the afore-mentioned operation.

Albany Hospital Nos. 6756-32 and 8462-32. The patient, aged 51 years, complained of pelvic pain radiating down both legs, especially the right one, and uterine bleeding of 4 weeks duration. A biopsy resulting in a diagnosis of carcinoma had been made in a hospital in another city 2 weeks before. The condition was considered to be inoperable at that time. On examination at the Albany Hospital, Sept. 21, 1932, the vaginal canal would just admit one's forefinger (hymen thick and rigid). One could feel that the cervical canal was dilated by a friable growth. On rectal examination the uterus was found to be enlarged and fixed in the pelvis with induration on both sides. Under gas oxygen anesthesia, Sept. 23, 1932, the cervix was exposed and an attempt was made to ascertain the origin of the tumor and its extent. This was only partially successful. It was our impression that while it might have arisen from the mucosa of the cervical canal, it was more likely a carcinoma of the body of the uterus which protruded into the cervical canal. Tissue was obtained for microscopic examination. A small nodule in the vaginal wall below the cervix was excised, thinking it might be a metastasis. It proved not to be one. One hundred milligrams of radium, in one capsule, was introduced through the cervix into the tumor and left in place for 24 hours. The posterior portion of the hymen was found to be torn through into the vagina as a result of the dilatation necessary to expose the cervix. No at-

tempt was made to close this wound but it was packed with gauze to control the bleeding. One hundred milligrams of radium was again inserted in the uterine cavity, Sept. 28, 1932, this time in two capsules of 50 mg. each, tandem formation. The patient was seen again Nov. 28, 1932. She considered herself well; both the pain and bleeding had ceased. A check-up was made in the Albany Hospital Nov. 29, 1932. On exposing the vaginal orifice a small red papilloma was noted at the junction of the posterior vaginal wall with the vulva — the site of the wound of the hymen caused 2 months before. This was excised, believing that while it might be malignant, it was more likely a tuft of granulation tissue which often appears in vaginal wounds that have not completely healed. A curettage was done and carcinoma was not found in the uterus. The uterus seemed smaller and less fixed.

The microscopic examination of the papilloma removed (see Fig. 36, with its legend) demonstrated that it was a papillary adenocarcinoma of the same histological structure as the primary uterine growth. The evidence presented, as well as its histological structure, indicates that it was a metastatic carcinoma of the vagina secondary to that of the uterus, probably caused by the transplantation of cancer cells in the wound of the vagina created two months before.

CASE 6. Advanced adenocarcinoma of the body of the uterus with metastases to the distal end of the left tube, the mucosa of the cervix and the anterior vaginal wall. This case was reported in a previous paper ⁴ (see Case 1 of that paper).

Albany Hospital No. 89234. The patient, aged 62 years, complained of uterine bleeding which had been more or less constant for 12 years. She had been curetted a year before I saw her. A diagnosis of malignancy was made at that time and the condition was considered inoperable. Since then she had had repeated X-ray treatments. At operation, March 16, 1923, the uterus was found to be enlarged, due to multiple small myomata and an extensive carcinoma. There was no gross evidence of metastases found at operation, other than a thickening of the fimbriated end of the left tube and a nodule in the anterior vaginal wall beneath the urethra. The entire uterus and both tubes and ovaries were removed. The metastasis in the anterior vaginal wall was excised and the vagina treated with radium. The patient made an uninterrupted recovery, has been seen by me several times since then, the last time in June, 1933. At no time have I been able to detect any evidence of a recurrence. Ten years have elapsed since her operation.

A study of the specimen removed (see Figs. 38 to 42 inclusive of present paper, with their legends, as well as the previous report of this case) demonstrated: (a) an advanced carcinoma of the body of the uterus, which in several places had extended almost entirely through the wall of the uterus to its serosa; (b) the presence of judged

emboli of cancer cells in veins in the peripheral zone of the uterus and in a vein in the tubal wall; (c) a carcinoma of the distal end of the left tube, which was judged to be of implantation origin; (d) clumps of cancer cells lying free in the lumen of the left tube and the judged implantation of one of these clumps in the tubal mucosa; (e) a metastatic carcinoma of the cervical mucosa judged to be of implantation origin; (f) a metastasis in the anterior vaginal wall which, from its histological structure, could have been primarily a surface implant, or a lymphatic, or venous metastasis. Carcinoma was not found in the ovaries or in the mucosa of the other tube. The finding of judged cancer cells in the veins of the peripheral zone of the uterine wall and in a vein of the tubal wall might be considered as evidence favoring the origin of the above described metastases through these channels. These may have been of more recent origin, or even caused by the trauma of the operation. The vaginal metastasis is the only one suggesting a possible origin from this source.

CASE 7. Adenocarcinoma of the body of the uterus with multiple carcinomas of the mucosa of the right tube, of various sizes, thus suggesting different ages.

Albany Hospital No. 890-29. The patient, aged 53 years, complained of uterine bleeding of 7 months duration. At operation, Feb. 9, 1929, which had been preceded by a diagnostic curettage, the uterus was found to be slightly enlarged. There was no gross evidence of metastases. The left tube and ovary appeared normal. The right tube was dilated and its fimbriated end occluded and adherent to the right ovary. The entire uterus and both tubes and ovaries were removed. The patient made a satisfactory convalescence and has remained well; was last seen in June, 1933.

Carcinoma was not found in the left tube and ovary. The data, supporting the theory that the multiple carcinomas of the mucosa of the right tube primarily arose from the implantation of cancer cells escaping from the uterine growth through the uterine ostium of the tube, are presented in Figs. 44 to 47 inclusive.

CASE 8. Advanced papillary adenocarcinoma of the body of the uterus, with extension through the wall of the uterus near the level of the internal os almost to its serosa. Carcinoma of the fimbriae of the left tube.

Albany Hospital Nos. 7001-30 and 229-33. The patient, aged 55 years, complained of uterine bleeding of 3 years duration. At operation, Sept. 27, 1930, the uterus was found to be about four times its normal size, due to multiple

myomas; the tubes and ovaries were essentially normal, save for adhesions about the fimbriated end of the left tube. In attempting to deliver the uterus, after ligating and severing the ovarian vessels and round ligaments, it was torn across at the level of the internal os, due to friability of the uterine wall at this level from the extension of the carcinoma almost to the serosa. The body of the uterus and both tubes and ovaries were removed and radium was applied to the cervical stump. The patient made an uneventful convalescence but eventually died of carcinoma in June, 1933.

A study of the specimen removed showed an advanced papillary adenocarcinoma of the body of the uterus. Carcinoma was not found in the right tube or ovary. Carcinoma having the same histological structure as that of the uterus was present in the fimbriae of the left tube (see Figs. 48, 49 and 50). It was not found elsewhere in the tube. The very superficial character of the growth would apparently exclude a metastasis through the lymph or blood stream. Its histological appearance suggests either an implantation or multicentric origin. Adhesions were found about the fimbriated end of the left tube at the operation, thus suggesting that something had escaped through the ostium of the tube into the peritoneal cavity. This favors the implantation theory.

CASE 9. Adenocarcinoma of the body of the uterus associated with multiple superficial carcinomas of both tubes.

Albany Hospital No. 6707-28. The patient, aged 54 years, complained of uterine bleeding of 7 months duration. At operation, Nov. 3, 1928 (no preliminary curettage), the uterus was found to be of normal size; bilateral hematosalpinx was present, but the ovaries appeared normal. There was no gross evidence of metastases. The appendix, entire uterus and both tubes and ovaries were removed. The patient made a satisfactory convalescence, felt well for several months after the operation, but later died of carcinoma.

On incising the uterus, after its removal, a superficial growth was found replacing the greater portion of the mucosa of the fundus, including that of both cornua. The tubes presented occluded fimbriated ends, dilated lumina and multiple papillary tumors of various sizes scattered over their lining. The data, supporting the theory that the tubal carcinomas primarily could have arisen from the implantation of cancer cells escaping from the uterine tumor through the uterine ostia of the tubes, are presented in Figs. 51 to 58 inclusive.

CASE 10. Multiple superficial papillary adenocarcinomas of the body of the uterus, associated with multiple papillary adenocarcino-

mas of the left tube and a very early squamous cell carcinoma of the vaginal portion of the cervix.

Albany Hospital No. 3760-30. The patient, aged 68 years, complained of uterine bleeding of over a year's duration. At operation, May 29, 1930 (preceded by a preliminary diagnostic curettage), the uterus was found to be of normal size; the left tube was dilated as in hydrosalpinx; the right tube and ovary appeared normal. There was no gross evidence of metastases. The entire uterus, both tubes, ovaries and appendix were removed. The patient made an uneventful recovery and has remained well.

On incising the uterus after its removal multiple superficial papillomas, of different sizes, were found scattered over the mucosa of the body of the uterus, including both cornua and the internal os. They were slightly more numerous in the left side of the body of the uterus and seemed larger in this situation. Similar papillary growths were found scattered over the mucosa of the dilated left tube (Fig. 59), the fimbriated end of which was occluded, but whose isthmus was patent. Carcinoma was not found in the right tube or in either ovary. The data, supporting the theory that the multiple carcinomas of the tubal and uterine mucosae arose from the implantation or grafting of cancer cells on the mucosa of these organs, are furnished in Figs. 59 to 65 inclusive. The possible escape of cancer cells from the uterine growth into the lumen of the left tube, through its uterine ostium, is indicated in Figs. 66 and 67. The reason for the failure of a similar phenomenon in the right tube is indicated in Fig. 68. The presence of a very early squamous cell carcinoma of the cervix (Fig. 69) gives support to the multicentric theory for the pathogenesis of the multiple carcinomas in all the different situations, but does not prove it.

CASE 11. Adenocarcinoma of the right cornu of a markedly bicornuate uterus, associated with multiple adenocarcinomas of the fimbriae and ampulla of the right tube, and carcinoma of the adherent right ovary.

Albany Hospital No. 94611. The patient, aged 56 years, complained of uterine bleeding. At operation, Dec. 27, 1923, (no preliminary diagnostic curettage), a bicornuate uterus was found with marked enlargement of the right cornu. The right tube was distended as in hydrosalpinx and fused by its occluded fimbriated end with the right ovary. The left tube and ovary appeared normal. There was no gross evidence of metastases or extension of the growth beyond the uterus. The appendix, entire uterus, and both tubes and ovaries were removed. The patient made an uneventful recovery but later died of carcinoma.

On incising the uterus a large growth was found confined to the right cornu (see Fig. 70). The microscopic study of sections of the uterus, right tube and ovary (see Figs. 71 to 77 inclusive, with their legends) demonstrated: (a) fragments of the carcinoma in the uterine ostium of the right tube; (b) carcinoma in newly formed tissue between the right ovary and occluded fimbriated end of the tube, which was fused with the surface of the ovary; (c) the invasion of both the ovary and the wall of the tube by carcinoma apparently starting in the opposed surfaces of the two organs; and (d) multiple carcinomas of different sizes in the mucosa of the tube. The escape of cancer cells from the uterine tumor, out through the lumen of the tube and its fimbriated extremity, resulting in adhesions between the surface of the ovary and the fimbriated end of the tube, and subsequent infolding of the tubal fimbriae and growth of the carcinoma, could easily account for the pathological findings in this situation. The implantation of carcinoma in the mucosa of the tube would account for the multiple carcinomas, apparently of different ages, found in the ampulla of the tube.

CASE 12. Adenocarcinoma of the body of the uterus, with metastases to the left ovary and bottom of the cul-de-sac, with extension through to the posterior vault of the vagina.

Albany Hospital No. 108718. The patient, aged 50 years, complained of uterine bleeding. At operation, Nov. 21, 1925 (preceded by a diagnostic curettage), the uterus was found to be of normal size and in normal position. The right tube and ovary appeared normal. Two judged endometrial implants were present on the lateral surface of the left ovary. There was an indurated mass in the posterior cul-de-sac which, prior to the operation, was diagnosed as endometriosis with extension through to the posterior vaginal vault. The bottom of the cul-de-sac was obliterated by the fusion of the sigmoid with the posterior wall of the cervix, as often occurs in endometriosis in this situation. There were no other gross evidences of metastases. The appendix, both tubes and ovaries, as well as the entire uterus with the tumor in the cul-de-sac, were removed. After incising the tumor removed from the cul-de-sac it was judged to be carcinoma and not endometriosis. Therefore radium was introduced in the vaginal vault. The patient had an uneventful convalescence, felt well for several months after the operation, but died Aug. 4, 1928, of carcinoma.

An adenocarcinoma of the uterus was found with deep invasion of its wall, but not through to its serosa. The tumor from the cul-de-sac, which was adherent to the posterior surface of the cervix, had the same histological structure as that of the uterine growth, but was not continuous with the latter. The judged endometrial implants of

the left ovary also proved to be adenocarcinoma. The distribution of the growth in the ovary and bottom of the cul-de-sac was similar to that often found in endometriosis. I believe that the carcinoma, in these situations, in this case was secondary to that of the uterus and might have arisen from cancer cells escaping through the lumen of the left tube (see also Figs. 78, 79 and 80 with their legends). Both tubes and the opposite ovary were studied and found free from carcinoma.

CASE 13. Large judged endometrial stromal cell sarcoma of the uterus associated with a superficial papillary adenocarcinoma of the uterine mucosa and a general peritoneal sarcomatosis including the omentum. This case was reported in a previous paper (Case 2 of that paper⁴).

Albany Hospital No. 87700. The patient, aged 54 years, complained of pain in the lower abdomen and uterine bleeding, the latter of over a year's duration. At operation, Dec. 6, 1922 (no preliminary curettage), the greatly thickened omentum was first observed and many peritoneal metastases were noted, as in peritoneal carcinomatosis of ovarian origin. The uterus was enlarged and a portion of its surface studded with metastases. The appendix, entire uterus and both tubes and ovaries were removed. On incising the enlarged uterus a large tumor was found, apparently arising from the mucosa of the anterior uterine wall. It presented the gross appearance of an edematous submucous myoma or sarcoma. The patient reacted badly after the operation and died on the fourth day.

At autopsy a chronic fibrous myocarditis was found; this and the peritoneal metastases were the only pathological conditions present. Carcinoma in lymph vessels was found only in the uterine cornu, where judged subperitoneal metastases were present (see Figs. 81 and 82 of present paper). The metastases of the ovaries and tubes were of a different type. They were more superficial and were situated in newly formed tissue on the surfaces of these organs (see Figs. 83 to 87 inclusive, with their legends). I believe that these latter metastases could have been of implantation origin from particles of the uterine tumor escaping through the lumina of the tubes into the peritoneal cavity. Fragments of the growth were found in the lumina of the tubes.

CASE 14. Advanced adenocarcinoma of the body of the uterus with deep invasion of the posterior uterine wall, but not through to its serosa, associated with: (a) three secondary carcinomas of the

mucosa of the left tube; (b) carcinoma of the fimbriae of the right tube; (c) general peritoneal carcinomatosis (including the ovaries); and (d) massive infiltration of the diaphragm, omentum and tissues of the posterior cul-de-sac with carcinoma. The retroperitoneal lymph nodes were not grossly malignant.

Albany Hospital No. 5493-30. The patient, aged 57 years, presented the physical signs of a peritoneal carcinomatosis of ovarian origin, and such was the preoperative diagnosis. Under spinal anesthesia, July 31, 1930, an exploratory incision was made to ascertain whether or not a major operation was indicated. The patient had difficulty in breathing and in spite of artificial respiration died in 3 hours.

The material for this study was obtained postmortem. The data, indicating that the carcinomas of the tubal mucosa may have resulted from cancer cells escaping into the lumina of the tubes and that the peritoneal carcinomatosis (including the ovaries) primarily arose from cancer cells escaping through the tubes into the peritoneal cavity, are presented in Figs. 88 to 105 inclusive, with their legends.

CASE 15. Adenocarcinoma of the body of the uterus, with peritoneal carcinomatosis restricted to the pelvic structures and the omentum; subsequent metastasis in the scar of the abdominal incision.

Albany Hospital Nos. 111-33, 1617-33 and 4788-33. The patient, aged 67 years, complained of uterine bleeding of 4 weeks duration. She was first admitted to the Albany Hospital, Jan. 5, 1933. A friable, necrotic, cauliflower type of growth was found distending the cervical canal. Biopsy showed it to be an adenocarcinoma of both glandular and solid type. It was impossible at that time to determine whether it began in the mucosa of the cervical canal or had extended down through the cervical canal from the body of the uterus. One hundred milligrams of radium in three capsules, tandem formation, was intruded through the cervix into the uterine cavity. The radium was left in place for 37½ hours. This was associated with a febrile reaction. She was readmitted for a check-up 2 months later. She felt well, her general health having improved greatly. The uterus was freely movable, although induration could be felt in the posterior cul-de-sac. At operation March 3, 1933, metastases were found on the surfaces of both ovaries, the bladder, the vesico-uterine fold, about the right round ligament and in the posterior cul-de-sac. Several epiploical appendages of the sigmoid were fused with the peritoneal metastases in the posterior cul-de-sac. The distribution of the ovarian and peritoneal metastases presented an exact duplication of the lesions often encountered in peritoneal endometriosis. Both tubes appeared patent. The peritoneum of the posterior cul-de-sac in places contained blood pigment, thus suggesting that blood had previously

escaped into the peritoneal cavity. In addition, metastases were present in the omentum. The uterus, both tubes and ovaries were removed. The portion of the omentum containing carcinoma, and all detected peritoneal metastases, were excised. The patient had an uneventful convalescence.

On incising the uterus after its removal the cervical mucosa appeared normal. The uterine mucosa for the most part was smooth and appeared normal, although it was roughened in a few places. Carcinoma was not found in the cervical mucosa, but was present in the roughened areas of the uterine mucosa just mentioned. Carcinoma was not found in the uterine wall (many sections from many blocks) or in the lumina of the tubes. For other evidence, indicating that the metastases to the ovaries and peritoneum of the pelvic structures arose from the implantation of cancer cells escaping through the lumina of the patent tubes during the application of radium, see Figures 106 to 112 inclusive. The patient was examined July 7, 1933, and a small nodule was noted beneath the skin of the scar of the abdominal incision. On bimanual examination marked induration was detected in the posterior cul-de-sac, indicating a further extension of the peritoneal carcinomatosis. The nodule in the abdominal scar was excised and proved to be carcinoma (Fig. 113) of the same histological structure as the primary uterine tumor and that of the peritoneal carcinomatosis observed at operation four months before. The strongest possible circumstantial evidence indicates that this metastasis arose from the successful implantation of particles of cancer in the wound of the abdominal incision at the previous operation.

DISCUSSION

In this study an attempt was made to ascertain the pathogenesis of carcinoma of the tubes and ovaries secondary to that of the body of the uterus, and especially to determine whether or not the trans-tubal migration of particles of cancer plays an important rôle in the etiology of these secondary tumors. Aside from the purely scientific interest in such a study, it was hoped that information of value might be obtained which could be applied in the treatment of carcinoma of the body of the uterus.

Purposely only those cases of carcinoma of the uterus associated with carcinoma of the tubes or ovaries were chosen to be presented, in which the primary growth was judged to be in the uterus, since those in the tubes and ovaries were less extensive and apparently of

more recent origin. In other cases which I have studied the primary carcinoma was judged to be in the ovary. In still others I was unable to form any opinion of the relation between the growths in the different organs and believed that some of these might possibly be instances of carcinoma of multicentric origin.

Thirteen cases of judged secondary carcinoma of the tubes, ovaries or both organs, of uterine origin, have been presented. Carcinoma was found in both the tube and ovary in six cases, only in the tube or tubes in five and only in the ovary in two. In addition, material from seven other cases of carcinoma of the body of the uterus was utilized because it presented features of importance in the study of the pathogenesis of secondary carcinoma of the tubes and ovaries, of uterine origin.

The following ways may be considered by which carcinoma of the tube may arise from that in the body of the uterus:

1. The continuous extension of carcinoma, arising in or later occupying the uterine cornu, through the uterine ostium of the tube by replacing the tubal mucosa, just as it often replaces the uterine mucosa; this might be designated as surface extension or replacement.
2. The invasion of the wall of the uterine portion of the tube by carcinoma that has invaded the wall of the uterine cornu through which the tube passes.
3. Continuous lymphatic permeation or metastasis through the lymph vessels of the mesosalpinx, and thence in a retrograde course through the tributary lymph vessels of the tube. Probably lymphatic permeation and metastasis also occurs through subperitoneal lymphatics of the uterus and tube, without the passage of the carcinoma through the main lymph channels in the broad ligament.
4. Metastasis through the blood stream.
5. The implantation, on the tubal serosa, of cancer cells which have escaped into the peritoneal cavity either through the lumina of the tubes or from carcinoma that has penetrated the uterine wall and reached the surface of its serosa.
6. The grafting or implantation in the tubal mucosa of cancer cells that have escaped from the uterine tumor into the lumen of the tube.
7. The differentiation of tubal epithelium into carcinoma caused by some agent escaping from the uterine growth into the lumen of the tube.

Carcinoma of the body of the uterus, arising in one cornu or extending to it, frequently passes through the uterine ostium of the tube by surface extension. Instances of this phenomenon are well shown in Figures 27, 28, 29, 30, 54 and 66. Cells, from the growth in this situation, may readily escape into the lumen of the tube, especially if the lumen of its uterine ostium is occluded by the growth (see Figs. 33, 34, 35 and 72). Although I have never observed the continued surface extension of carcinoma of the uterine ostium of the tube out through its entire uterine portion into the isthmus, I believe that it may occur. Blood containing cancer cells also may escape through a patent uterine ostium of the tube from uterine carcinoma not occupying the uterine cornu.

I have not observed the invasion of the wall of the uterine portion of the tube by carcinoma in the wall of the uterine cornu about it in the same manner as the uterine wall itself is invaded, or as structures becoming adherent to the wall of the uterus, at the site of its penetration by carcinoma, are invaded (compare Fig. 9 with Fig. 2).

Lymphatic permeation with or without embolic metastases was responsible for two instances of carcinoma of the tubes, secondary to that of the uterus in this series (see Cases 3 and 4, and Figs. 18, 19, 22, 23 and 24).

In another instance (Case 2) two judged subperitoneal and one submucosal metastasis of the tube were found, which may or may not have arisen from cancer cells carried through the lymph vessels (see Figs. 10, 12 and 13).

I have observed the invasion of the venous sinuses of the uterine wall by carcinoma of the body of the uterus and seen judged cancer emboli in the lumina of these sinuses, but have been unable to determine the part played by the blood stream in the pathogenesis of carcinoma of the tubes and ovaries, secondary to that of the body of the uterus. Possibly some of the metastases shown in Figures 1, 3, 7, 10, 11, 12, 81, 82 and others may have arisen from cancer cells carried through the veins and not the lymphatics. The invasion of the lymphatics about these metastatic tumors does not prove the pathogenesis of the latter. A secondary tumor, irrespective of its pathogenesis, apparently determines its own methods of invasion and dissemination, depending upon its inherent traits, its situation and the structure of the organ in which it is located. The invasion of the venous sinuses of the uterine wall is probably of frequent occurrence in car-

cinoma of the body of the uterus. I believe that the blood stream may play a more important rôle in the etiology of metastases to the tubes and ovaries than I have determined.

Metastases of the serosa of the tubes, as a part of an extensive peritoneal carcinomatosis (sarcomatosis in one instance), was found in two cases (Cases 13 and 14), see Figures 86, 87, 88, 93, 99 and 100. There was strong circumstantial evidence in each instance that the growth first gained access to the peritoneal cavity by the transtubal route.

In the largest and most interesting group (consisting of seven cases, Cases 6, 7, 8, 9, 10, 11 and 14, with carcinoma in both tubes in one case) the carcinoma of the tubes seemingly must have arisen either from a differentiation of tubal epithelium or from cancer cells grafted in the tubal mucosa. In only one case (Case 8) was a single carcinoma found in the tube. In all the others the tumors were not only multiple but varied in size, thus suggesting different ages. They were all superficial. In each instance there was strong evidence that cancer cells could have escaped into the lumen of the tube from the primary uterine tumor, as into the lumen of a lymph vessel. In five of the seven cases the fimbriated end of the tube was occluded. In all of these cases (Cases 6, 7, 9, 10 and 11) carcinoma was present in the uterine cornu of the affected tube (both tubes in one case) with evidence that it had or might have invaded the uterine ostium of the tube (see Figures 47, 54, 55 and 66). Grossly the isthmus of the tube in each case appeared normal. The microscopic examination of the uterine portion of four of these tubes failed to show complete occlusion of any of them, other than that caused by the extension of carcinoma about the uterine ostium into the lumen of the tube. In three instances (Figs. 56, 67 and 72) clumps of cancer cells were found in the lumen of the tube distal to the growth filling the uterine ostium. I believe that the closure of the fimbriated end of the tube, at least in some instances, might have resulted from the development of carcinoma in the tubal mucosa. The possibility of this phenomenon is suggested in Figure 40.

When the fimbriated end of the tube is closed and the uterine ostium plugged with carcinoma a condition of hydro- or hematosalpinx arises. Since the mucosa is stretched under these circumstances injury to its epithelial covering might occur more readily than when the tube is not dilated. In this way conditions more favor-

able for the secondary grafting of cancer cells floating about in the lumen of the tube might be created. These secondary implantations of carcinoma might arise, either from carcinoma established in the tube, or from the repeated escape of cancer cells from the growth situated in its uterine ostium. The data supporting the implantation theory in the above cases are: (a) the tubal carcinomas are superficial in character, multiple and of different sizes; (b) the evidence that cancer cells from the advancing (and therefore the growing) part of the tumor could well have been discharged into the lumen of the tube; and (c) finally the similarity in histological structure of the carcinomas of the tubal mucosa and the implantation carcinomas on the lining of lymph vessels and on the peritoneum. I do not see how the multicentric theory can be applied as logically in the cases just reported, unless the agent causing the differentiation of tubal epithelium into carcinoma is derived from something escaping from the uterine growth into the lumen of the tubes: if it is true here it might also be applied to explain the pathogenesis of similar lymph vessel and peritoneal metastases of accepted implantation origin.

Carcinoma of the ovary was found in eight of the thirteen cases and in only two cases without carcinoma of the accompanying tube. In one instance the growth had invaded the ovary through the utero-ovarian ligament from the continuous extension of the carcinoma that had penetrated the uterine wall (Fig. 14).

In two other instances (Figs. 18 and 25) the ovary was invaded from within by a continuous permeation of the lymph vessels or permeation with metastases. A similar phenomenon was present in the tubes accompanying the ovaries.

In five cases (Cases 11, 12, 13, 14 and 15) the ovarian carcinomas (multiple in each instance) were superficially situated and were either on the surface of the ovary (Figs. 83, 84, 85, 94, 95, 98, 101, 103, 104, 106 and 107) or had apparently invaded the ovary from its surface (Figs. 73, 74, 78 and 80). Two of these were associated with multiple carcinomas of the tubal mucosa (Cases 11 and 14). In one of the latter (Case 11) peritoneal carcinomatosis was not present other than that in adhesions between the surface of the ovary and the occluded fimbriated end of the tube. In the other there was an extensive peritoneal carcinomatosis. In another case (Case 13) an extensive peritoneal sarcomatosis was present with implantations on the serosa of the tube, but without metastases to the tubal mucosa.

I believe that the transtubal migration of cancer cells from a uterine growth played a very important rôle in the etiology of the cases of judged secondary carcinoma of the tube or ovaries, or both organs, just reported, namely, in nine of the thirteen cases. I do not desire to create the impression that the transtubal migration of cancer cells in either direction necessarily plays as important a rôle in the pathogenesis of all cases of carcinoma in these different organs as it did in the cases just reported.

In two cases of this series (Figs. 37 and 59) judged implantation of carcinoma in the endometrium is described secondary to a primary carcinoma of the body of the uterus. I believe that this is of rare occurrence. I have been looking for it since I encountered Case 10, three years ago, and have encountered only one other instance of multiple carcinomas of the uterine mucosa and that is shown in Figure 37.

In Figure 39 is shown a judged implantation of uterine carcinoma on the mucosa of the cervix. It is the only instance of the kind that I have found, and I have been looking for it ever since I encountered this one ten years ago. I have been greatly impressed with the rarity of the involvement of the cervical mucosa in carcinoma of the body of the uterus, even in advanced cases.

In Figure 36 is shown a judged implantation of uterine carcinoma in the scar of a vaginal wound. On the other hand, the pathogenesis of the vaginal metastasis shown in Figure 38 is more difficult to state. I have been greatly interested in the etiology of the vaginal metastases found in carcinoma of the body of the uterus, but have been unable to make a satisfactory study of this condition. The majority of patients with carcinoma of the body of the uterus are past the menopause. Senile changes in the vaginal mucosa, often with superficial ulcerations, are of frequent occurrence in women of this age. It occurred to me that these superficial ulcerations might furnish favorable soil for the grafting of any viable cancer cells escaping through the cervical canal. In spite of my enthusiasm over the implantation of carcinoma, it has been my impression that in the majority of the instances of vaginal metastases that I have encountered the lymphatic or venous route offered a more rational explanation of their pathogenesis. This is particularly true when additional metastases are found elsewhere than in the vagina.

An instance of metastasis in the scar of the abdominal incision was

encountered in this series. The circumstantial evidence leading to the development of this metastasis indicates that it arose from the implantation of cancer cells during the operative removal of the uterus, tubes and ovaries and the excision of peritoneal implantations four months before. At the time of the operation there were no visible metastases in the peritoneum of the abdominal wall (see Case 15, and Fig. 113). This is another contribution to the implantation of carcinoma. The fundamental stages in this process are the same in all instances, namely, the lodging of cancer cells in a wound, however created, with the attempted healing of the wound and the continued growth of the cancer cells in their new situation.

The conditions found in this small series of cases supports the stand taken by those who believe that carcinoma of the body of the uterus, when feasible, should be treated by the removal of the entire uterus and *both tubes and ovaries*. It condemns the routine treatment of carcinoma of the body of the uterus with radium, since it cannot be expected to reach the possible secondary cancers in the tubes and ovaries. Also the introduction of radium into the uterine cavity is associated with the danger of disseminating blood containing particles of cancer through patent tubes into the peritoneal cavity (see Case 15, and Figs. 106 to 112). Radium should be used only in poor operative risks, or after the uterus has been removed.

The operative removal of advanced carcinoma of the body of the uterus is associated with a relatively high primary mortality and a low percentage of cures, yet three of these patients with secondary carcinoma in the tubes are living and clinically free from cancer — Case 6 after ten years, Case 7 after four years and Case 10 after three years.

CONCLUSIONS

By continuous extension, carcinoma of the body of the uterus invades the potential tissue spaces of the uterine wall and may even penetrate the entire wall, including its serosa. Pelvic structures frequently become adherent to the surface of the uterus thus penetrated by the growth and in turn are invaded by it. Circumstantial evidence indicates that cancer cells may escape into the peritoneal cavity from a growth that has reached the surface of the uterus before it became adherent to nearby structures, and may give rise to implantation peritoneal carcinomatosis.

Both the lymphatics and venous sinuses of the uterus are invaded by the continuous extension of carcinoma. Secondary carcinoma of the tubes and ovaries arises from the continuous permeation of the lymphatics and also from cancer emboli. Circumstantial evidence indicates that cancer cells become implanted on the endothelial surface of lymphatics just as similar evidence indicates that cancer cells escaping into the peritoneal cavity, from ovarian carcinoma, become implanted on the surface of the peritoneum.

By the continuous extension of carcinoma of the body of the uterus the mucosa is replaced by the growth, and when situated in a uterine cornu it frequently extends through the uterine ostium of the tube and replaces the mucosa of the uterine portion of the tube. Particles of cancer are broken off from the growing (advancing) portion of the growth in this situation and as emboli migrate into the lumen of the tube beyond, as similar emboli migrate in lymph vessels and into the peritoneal cavity. Superficial carcinomas are found in the mucosa of the tubes, in their fimbriae, and on the ovary and peritoneum of patients with carcinoma of the body of the uterus. They present the same histological structure as recognized implantation carcinoma of the lining of lymphatics, and also on the peritoneum from ovarian carcinoma, with evidence that particles of cancer from the primary uterine growth could have reached the situation of these metastases through the lumina of the tubes. Therefore, I believe that their pathogenesis in many instances is the same; namely the lodging of the cancer cells on the surface of their host, injury to that surface already present or created by the carcinoma, the attempted repair of the injury with the continued growth of the carcinoma in this situation. In like manner particles of uterine carcinoma become implanted in the uterine and cervical mucosa, and in wounds of the vagina and of abdominal incisions.

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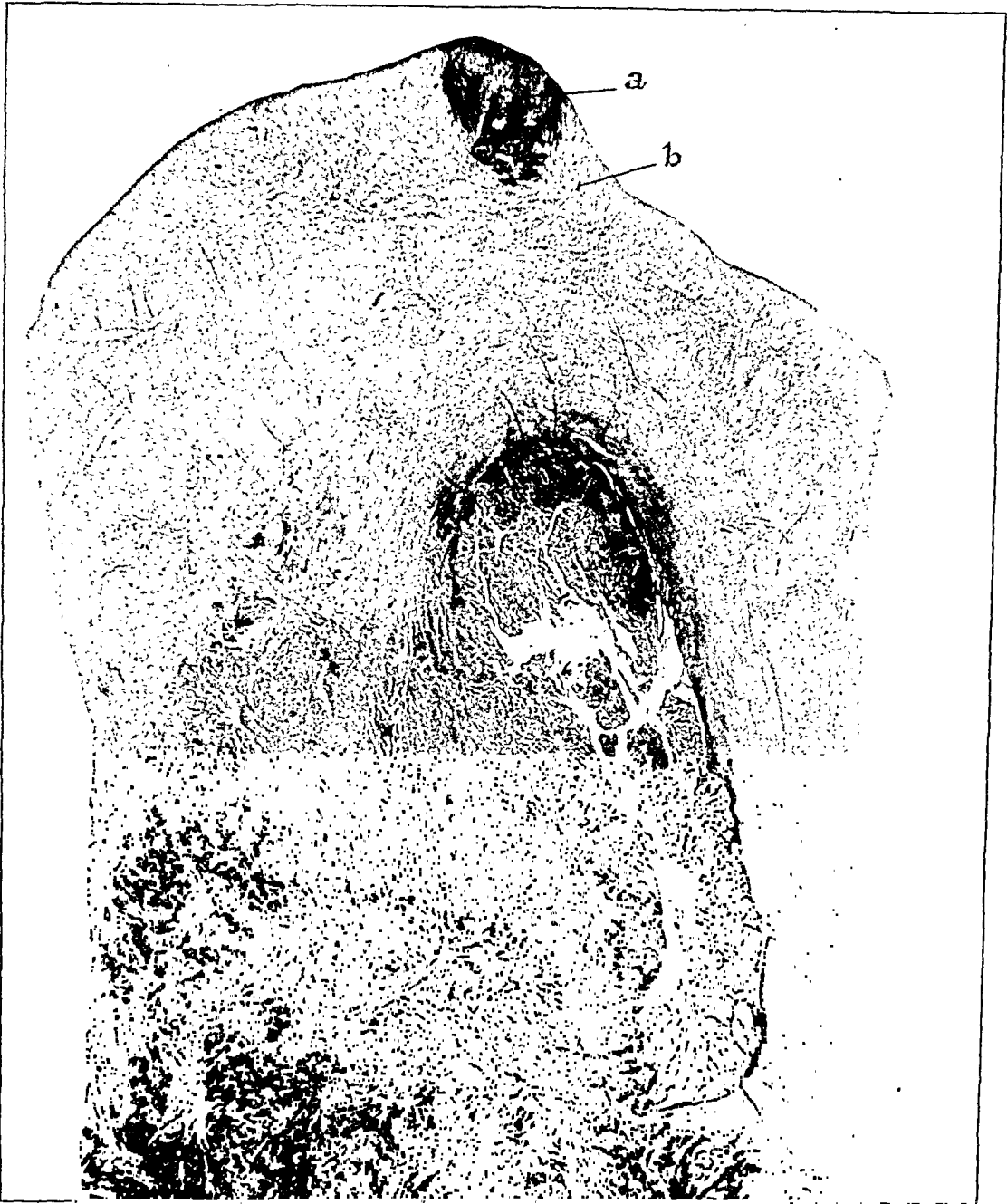
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DESCRIPTION OF PLATES

PLATE I

FIG. 1. Photomicrograph of a sagittal section of a portion of the left uterine cornu (Case 1). It shows well the invasion of the posterior wall of the uterus by the carcinoma. Here the growth is an adenocarcinoma with the solid phase predominating (see Fig. 5). The mucosa lining the anterior wall of the uterine cavity has been replaced by a papillary adenocarcinoma, without evidence of invasion of the uterine musculature. The variations in the histological structure of the growth in different portions of this specimen demonstrate the difficulty that may arise in typing adenocarcinoma of the uterus. A judged subperitoneal metastasis is indicated at "a" and an embolus of cancer cells in a lymphatic at "b." Many sections studied of this portion of the block failed to demonstrate that the carcinoma in the lymphatic "b" was continuous with the primary growth. Several other subperitoneal metastases were present in the left uterine cornu; for a higher magnification of one of these see Fig. 3. $\times 5$.

FIG. 2. Photomicrograph of a portion of the posterior uterine wall and the omentum that is fused with it (Case 1). The strands of carcinoma shown in this section are continuous with the primary growth indicated in the preceding illustration, and have penetrated the entire thickness of the posterior uterine wall. Cancer cells may have escaped into the peritoneal cavity before the omentum came to the rescue, the latter preventing the dissemination of cancer cells, just as it often checks the spread of bacterial infections. The omentum, (to the left) adherent to the posterior surface of the uterus, has been invaded by the growth. $\times 54$.



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PLATE 2

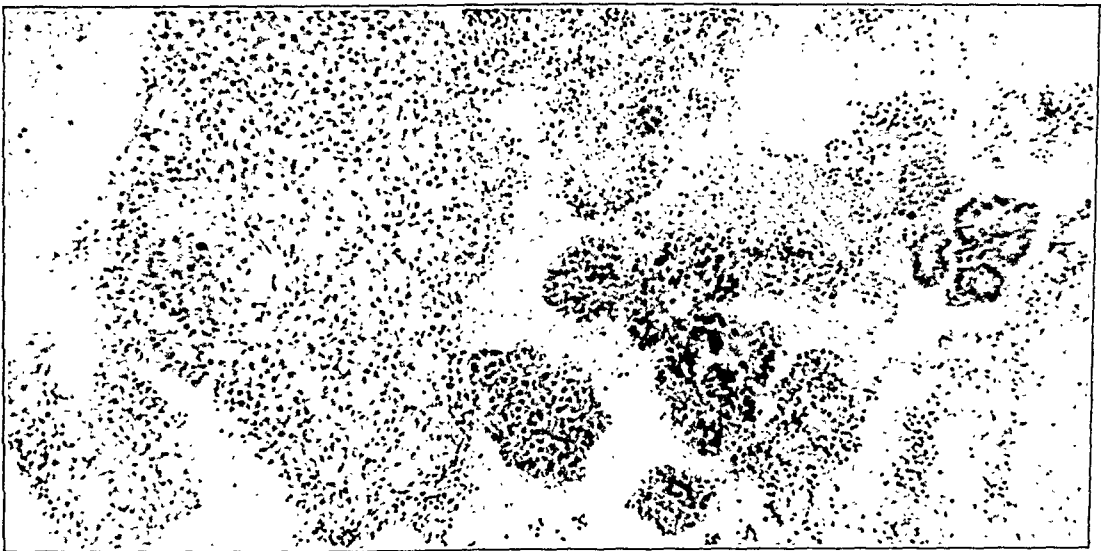
FIG. 3. Photomicrograph of a section through a judged subperitoneal metastasis of the left uterine cornu, similar to the one shown in Fig. 1, but smaller. To the left the carcinoma has not reached the peritoneum, but in the center and to the right it has. The condition present in the latter situations suggests that cancer cells might have escaped into the peritoneal cavity (see next illustration). It was impossible to determine the pathogenesis of this metastasis and the one shown in Fig. 1, as well as other similar ones present in this specimen. They were judged to be of lymphatic origin, the cancer cells having been deposited in the subperitoneal tissues with subsequent extension toward its surface. $\times 54$.

FIG. 4. Photomicrograph of a section of the sediment from centrifugalized blood-stained fluid obtained from the posterior cul-de-sac at the time of the operation. I believe that the large mass of faintly staining cells, to the left, is of mesothelial origin. In the center are clumps of hyperchromatic cells with variation in the size and staining qualities of their nuclei. These cells resemble those of the primary growth (see next illustration) more closely than they do the judged mesothelial cells. Clumps of these cells, to the right, are arranged in the form of glands. Mitotic figures are present. I am unable to assert that they are clumps of cancer cells and not differentiated mesothelial cells. If cancer cells they may have escaped from strands of carcinoma extending into the peritoneal cavity, from the direct extension of the growth through the uterine wall as suggested in Fig. 2, or from a similar phenomenon indicated in Fig. 3. On the other hand they may have escaped through the lumina of the tubes during the diagnostic curettage 6 days before. $\times 130$.

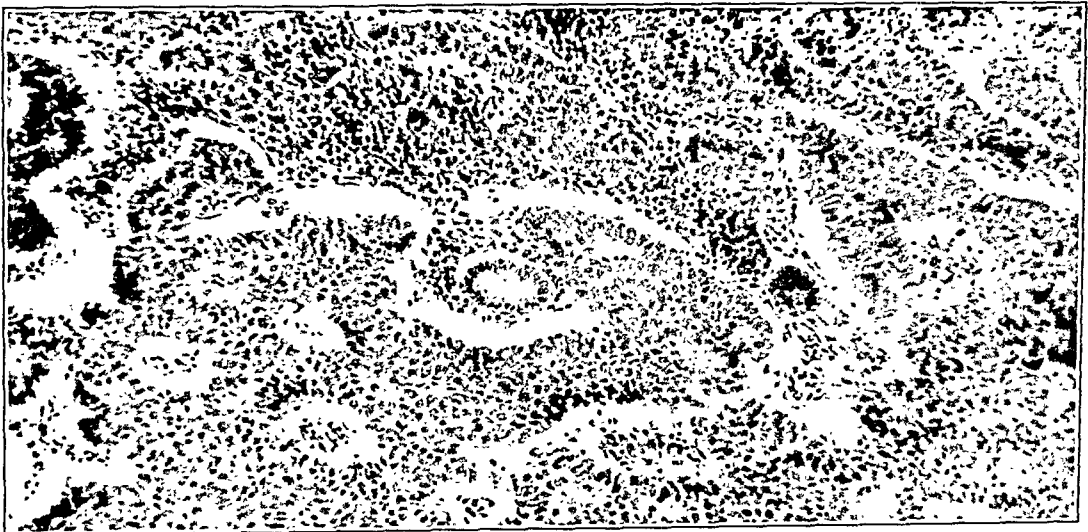
FIG. 5. Photomicrograph of the primary uterine tumor taken from the portion of the growth invading the posterior uterine wall, Fig. 1. It is an adenocarcinoma — solid phase predominating. The masses of cancer cells and the gland in the center closely resemble the clumps of hyperchromatic cells and the cells with glandular formation found in the sediment obtained from fluid in the posterior cul-de-sac shown in the preceding illustration. $\times 130$.



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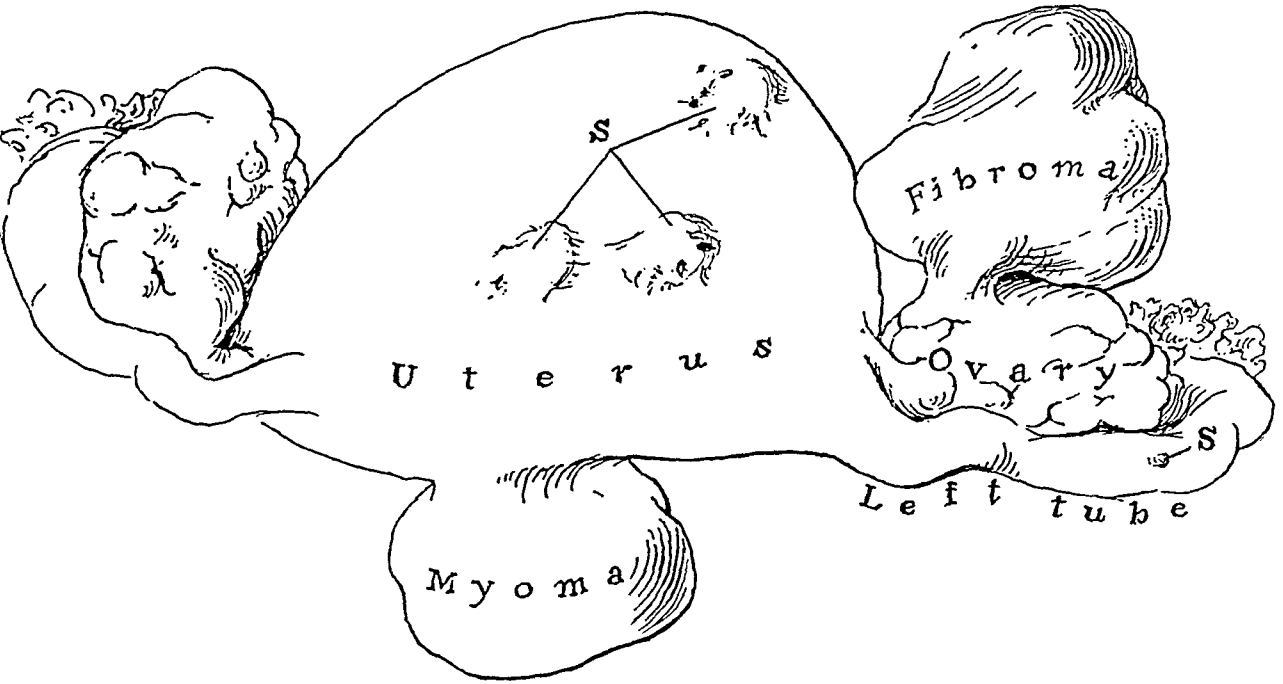
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PLATE 3

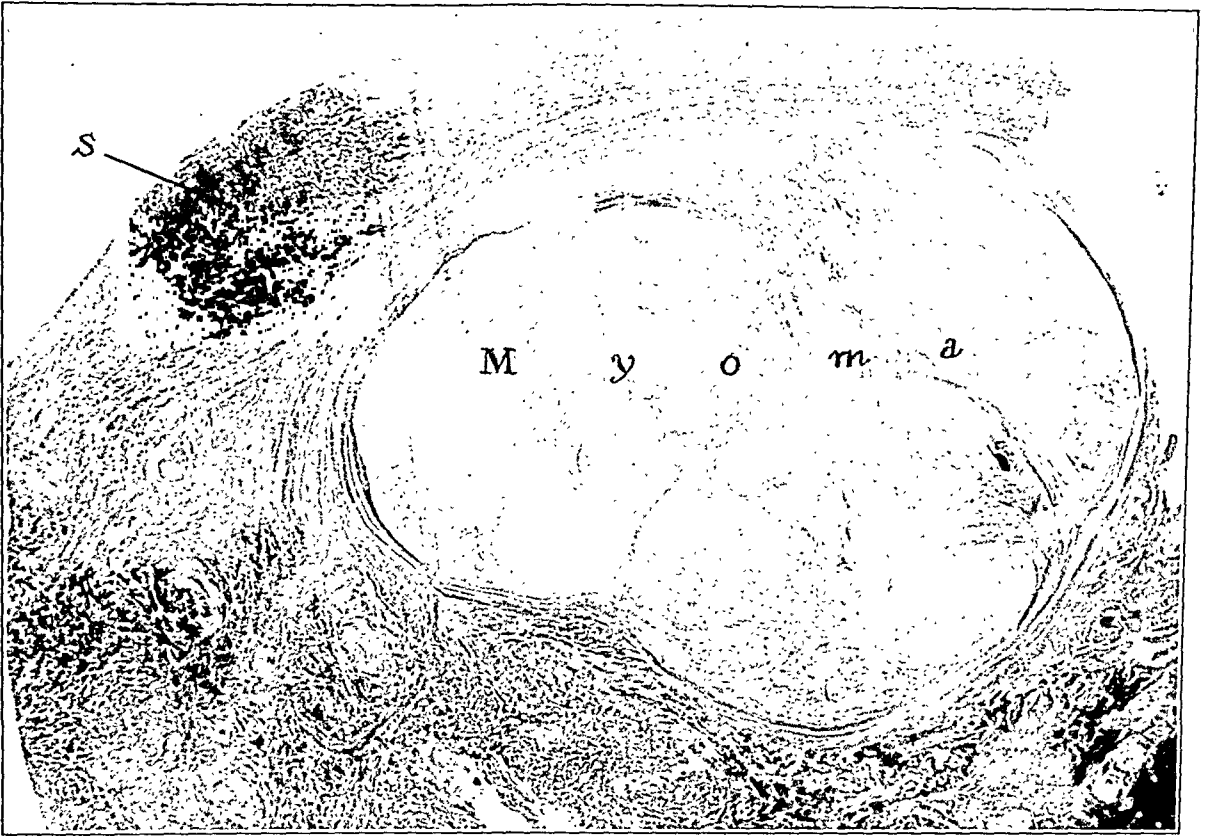
FIG. 6. Fundus of the uterus with the tubes and ovaries (Case 2). The uterus contained multiple small myomas and was adherent posteriorly to the sigmoid and the terminal loop of the ileum, due to the extension of the growth through the posterior wall of the uterus. Peritoneal carcinomatosis was also present in the posterior cul-de-sac. Slightly elevated scars (s) are present on the surface of the uterus grossly (and microscopically) similar to the subperitoneal metastases in the left uterine cornu of the preceding case. The right tube and ovary are normal. A pedunculated fibroma is attached to the posterior surface of the left ovary, which has been invaded by the uterine carcinoma (see Fig. 14). The left uterine cornu is larger than the right, due to its infiltration with carcinoma (see Fig. 9). Two subperitoneal metastases are present in the ampulla of the left tube (one is shown at "s"). See also Figs. 10 and 12. Natural size.

FIG. 7. Photomicrograph of a cross-section of a portion of the posterior uterine wall through one of the scars shown in the preceding illustration. The uterine wall has been infiltrated by the direct extension of the primary growth (lower third of the photomicrograph). An intramural myoma is present — carcinoma was not found in it. A judged subperitoneal metastasis is indicated at "S" similar to the one shown in Fig. 1. Serial sections of this block were not made and therefore the origin of the growth in this situation by continuous extension from the primary tumor cannot be excluded absolutely. $\times 5$.

FIG. 8. Photomicrograph of a portion of the uterine wall infiltrated by the carcinoma. It is an adenocarcinoma with both glandular and solid arrangement of the cancer cells. $\times 54$.



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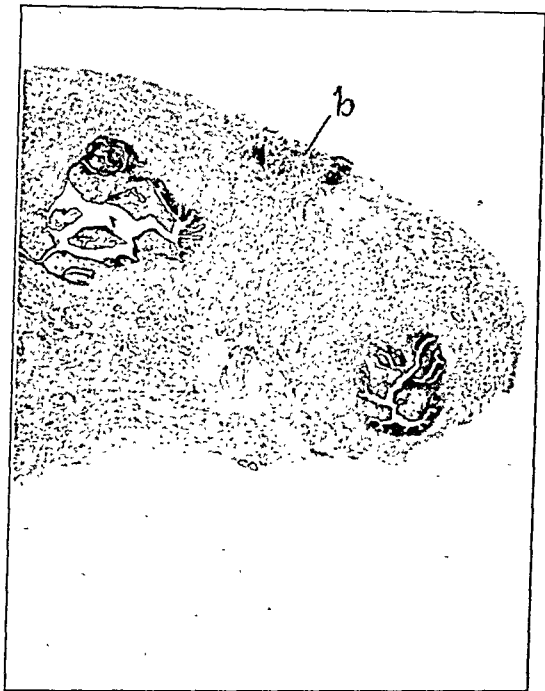
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PLATE 4

- FIG. 9. Photomicrograph of a section through the left cornu of the uterus. The uterine cornu is infiltrated by the continuous extension of the carcinoma. The tube, shown in cross-section, is surrounded by the growth, but is not actually invaded by it. Its lumen is patent and is lined by normal mucosa. At "a" and "b" the strands of carcinoma have extended through the peritoneum. $\times 10$.
- FIG. 10. Photomicrograph of a portion of two longitudinal sections (from different levels of the same block) of the distal end of the left tube. At "b" is a judged subperitoneal metastasis similar to the one shown in Fig. 7 (compare also with Figs. 11 and 12). At "a" is a judged submucosal metastasis in the fimbriae of the tube. Compare with Figs. 11, 12 and 13. $\times 5$.



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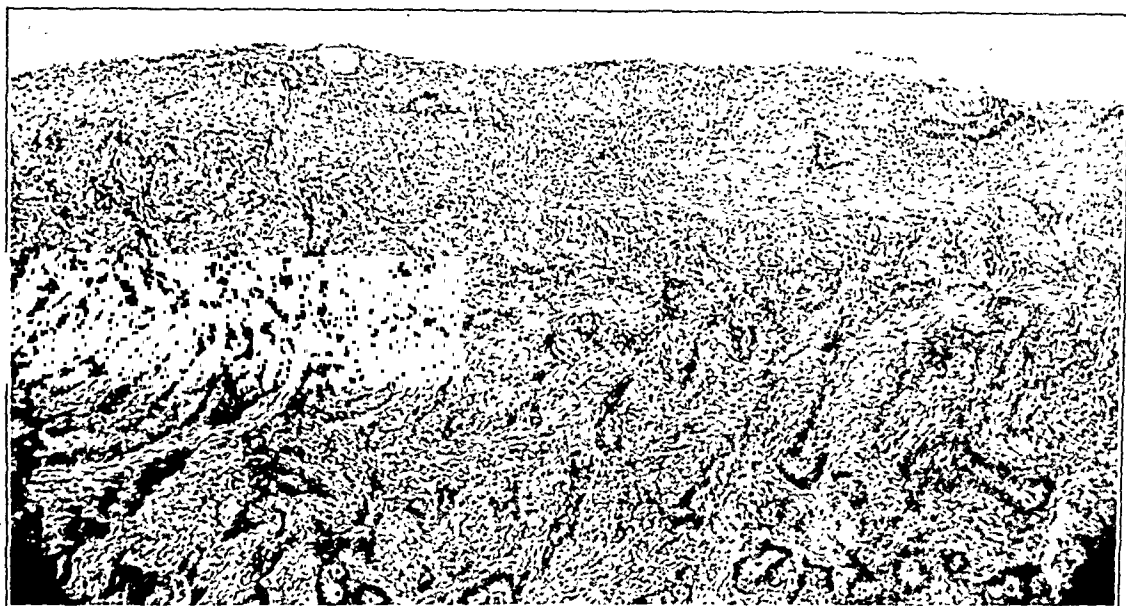
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PLATE 5

FIG. 11. Photomicrograph of a superficial portion of the judged subperitoneal metastases of the uterine wall shown in Fig. 7. The growth apparently started in the uterine wall and has extended through to the peritoneal surface. Compare with Fig. 3. In this situation the glandular phase of the carcinoma predominates. $\times 54$.

FIG. 12. Photomicrograph of the judged subperitoneal metastasis to the tubal wall indicated by "b" of Fig. 10. The general histological formation of this tumor is similar to that of the preceding one. It seems to me that the growth probably started in the deeper portion of the subperitoneal tissues and spread in all directions, even to the surface of the peritoneum. Others may claim that it arose from an activation and differentiation of the mesothelium (see arrow) or the implantation of cancer cells on the peritoneal surface of the tube. Still others may assert that the apparent extension of the carcinoma to the surface of the peritoneum is misleading, that what appears to be a direct extension of the growth to the surface of the peritoneum is in reality the downward growth of the activated mesothelium to meet the carcinoma. A careful study of the peripheral extensions of the growth shows that they are the same in all directions. $\times 54$.

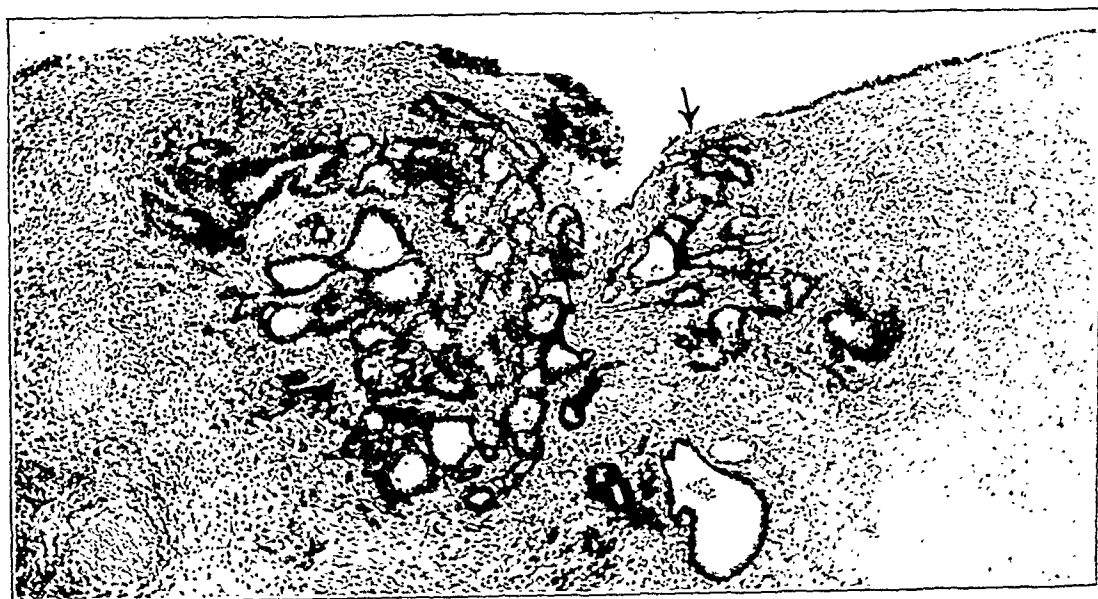
FIG. 13. Photomicrograph of the judged submucosal metastasis of the fimbriae of the tube shown in "a" of Fig. 10. If the growths shown in the preceding two illustrations are subperitoneal metastases why should not this one be a submucosal metastasis rather than a primary tumor in this situation, or an implantation? Its apparent origin from the epithelium (see arrow) may be misleading. In reality it may be the extension of the growth to the mucosal surface of the fimbriae as the carcinoma in the two preceding illustrations extended to the surface of the peritoneum. It was impossible to ascertain whether the cancer cells reached the situations indicated in these three metastases through the lymphatics or veins. With our present knowledge of the pathogenesis of similar metastases we believe that the lymphatics are the most likely route. $\times 54$.



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PLATE 6

FIG. 14. Photomicrograph of a section of the left ovary, the utero-ovarian ligament and a portion of the wall of the uterus (Case 2). By a continuous extension the carcinoma has penetrated the uterine wall, passed through the utero-ovarian ligament (see arrows) and invaded the ovary. At "a" the growth has entered the lumen of a vessel (see the next illustration). The continuous invasion of the ovary was demonstrated by serial sections of this portion of the block. At "b" the growth has extended through the uterine wall to the peritoneal cavity (see Figs. 16 and 17), just as it invaded the lumen of the vessel at "a." $\times 8$.



PLATE 7

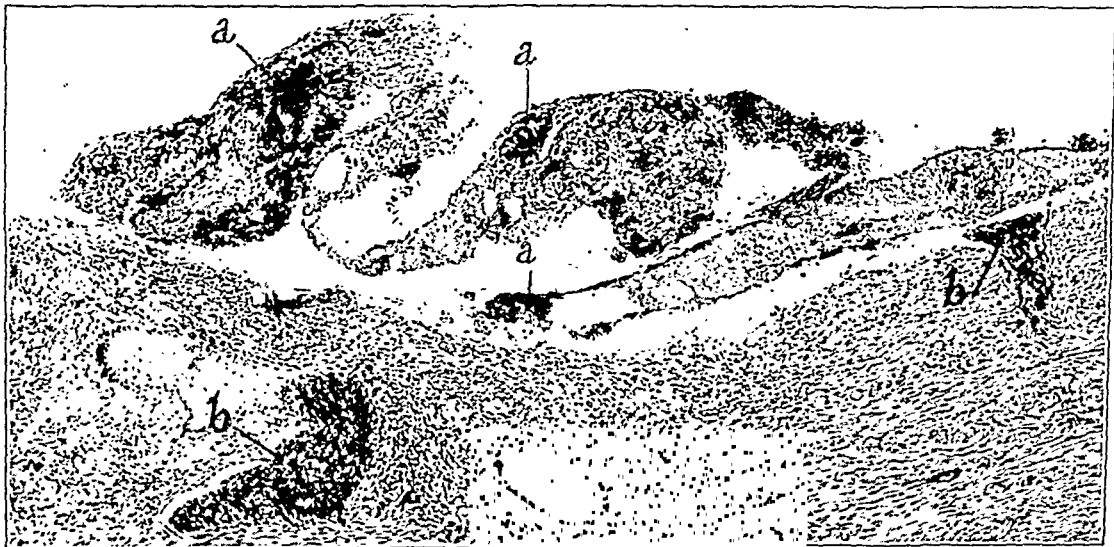
FIG. 15. Photomicrograph (higher magnification) of the area "a" of the preceding illustration demonstrating the extension of the carcinoma into the lumen of a vessel. It resembles a lymph vessel more than a vein, but a small amount of blood was present in its lumen. One can readily see that cancer cells could escape easily from the growth which has penetrated the lumen of the vessel and as emboli might be carried wherever the blood or lymph stream might take them. In this section the glandular phase of the carcinoma predominates. $\times 54$.

FIG. 16. Photomicrograph (higher magnification) of area "b" of Fig. 14. There has been a peritoneal reaction to some irritant with the development of newly formed tissue on the surface of the peritoneum. Enmeshed in this tissue are clumps of cells ("a," "a" and "a") histologically indistinguishable from the solid phase of the primary uterine growth, shown in Fig. 8, and also in the uterine wall beneath the newly formed tissue ("b" and "b"). Serial sections were made of this portion of the block to determine the relation between the carcinoma in the uterine wall and the clumps of judged cancer cells in the newly formed tissue above it. See the next illustration. $\times 54$.

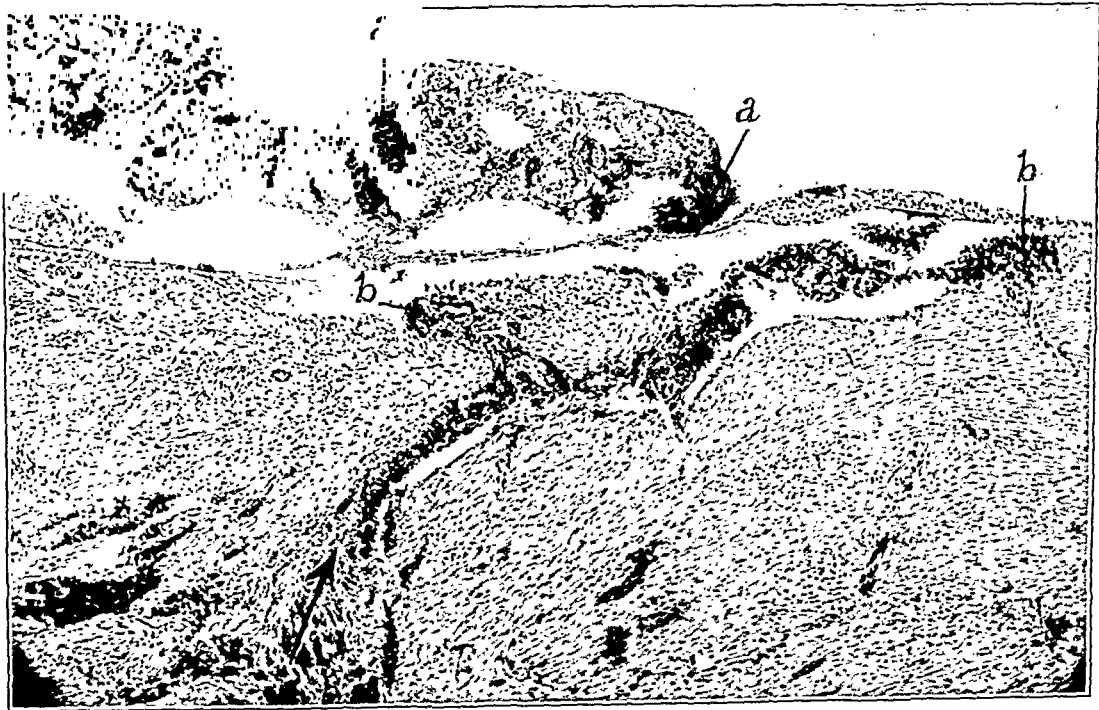
FIG. 17. Photomicrograph of a section from the same series as the one shown in the preceding illustration. The continuous extension of the carcinoma (see arrow) through the uterine wall to the surface of the peritoneum is well shown. In this section the solid phase of the tumor (shown in Fig. 8) prevails; the reaction of the peritoneum, attempting to check the further extension of the growth, is indicated by the newly formed tissue on the surface of the uterus, also shown in the preceding illustration. The clumps of judged cancer cells ("a" and "a") enmeshed in this newly formed tissue are not continuous with the cancer ("b" and "b"), which has penetrated the uterine wall. We have in this, and other sections from this block, the strongest possible circumstantial evidence that strands of carcinoma penetrating the uterine wall may give rise to implantation carcinoma of the peritoneum about the site of the penetration of the uterine wall by the growth. If here, why not also in the peritoneum of the posterior cul-de-sac where a peritoneal carcinomatosis was present? $\times 54$.



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PLATE 8

FIG. 18. Photomicrograph of a cross-section of the right tube, mesosalpinx and portion of the enlarged ovary from a patient with extensive carcinoma of the body of the uterus, secondary to that of the cervix (Case 3). In the gross specimen one could follow easily the subperitoneal lymphatics distended with cancer cells as with a white injection mass, an excellent example of lymphatic permeation. Note a large lymphatic "a" in the mesosalpinx lined with carcinoma. The lymphatics of the tube are also distended with carcinoma, most marked in the mucosa (see also the next illustration). The carcinoma of the ovary tends to form cysts. In both organs the growth has developed within the organ and bears no relation to their surfaces. One would not be tempted to attribute the conditions shown here to the trans-tubal migration of cancer cells. $\times 5$.

FIG. 19. Photomicrograph (higher magnification) of a portion of the mucosa of the tube shown in the preceding illustration. The lymphatics of the mucosal folds are distended with carcinoma. In one place "a" the growth has extended through the wall of a lymphatic and invaded the lumen of the tube. Its relation to tubal epithelium is well shown to the right of the letter "a." In some ways it, here, suggests that the growth might have arisen from the tubal epithelium. A fragment of carcinoma lying free in the lumen of the tube is indicated at "b." Such a fragment might escape into the peritoneal cavity. The conditions presented in these photomicrographs admit of only one rational interpretation, and that is that the carcinoma of the tube and ovary is secondary to that of the uterus by lymphatic permeation, or metastases, or both phenomena. $\times 54$.



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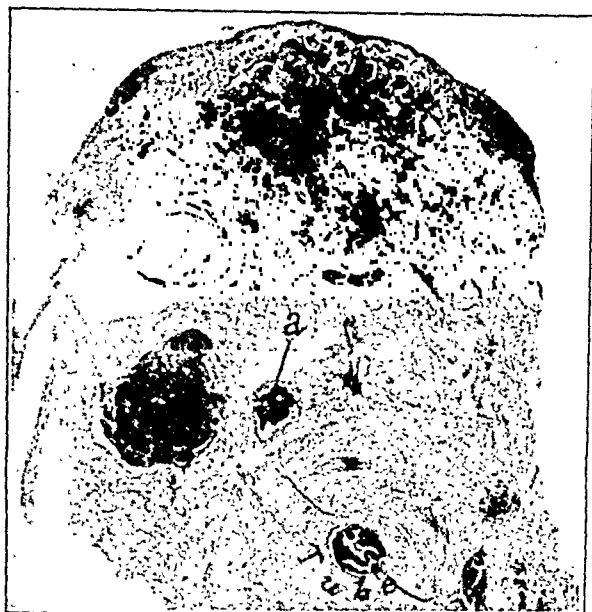
PLATE 9

FIG. 20. Photomicrograph of a section of the distal portion of the left uterine cornu, including the tube (Case 4). The uterine wall is infiltrated with carcinoma. The lumen of the tube is patent, but carcinoma is present in its mucosa, beneath the epithelium. At "a" an apparent implantation of carcinoma is shown on the lining of a lymph vessel. See next illustration. $\times 10$.

FIG. 21. Photomicrograph (higher magnification) of the lymph vessel indicated by "a" of the preceding illustration. When this implantation was found all sections of the block, which had been cut already, were stained and serial sections were then made of the rest of the block. We were unable to demonstrate that this apparent implant was continuous with carcinoma elsewhere. I believe that it is an implantation of carcinoma of the skin graft type occurring on the endothelial surface of a dilated lymph vessel, just as an ovarian carcinoma sometimes becomes implanted on the peritoneum. $\times 54$.

FIG. 22. Photomicrograph of a cross-section of the tube (Case 4). The patent lumen of the tube appears in the lower right portion of the photomicrograph. The wall of the tube is greatly thickened, due to lymphatic stasis, the result probably of the occlusion of efferent lymph vessels by carcinoma. Some of the dilated lymph vessels are almost completely filled with the growth, others are not. In places the carcinoma is attached to the wall of the lymph vessels, but in the majority of the lymph vessels the growth appears as clumps of cells (emboli) floating about in the lumen of the vessel or as cross-sections of strands of carcinoma. Serial sections were made to determine whether or not true emboli were present. We were able to demonstrate that at least some of the carcinoma in these dilated lymph vessels are true emboli and not long strands or threads (roots) continuous with the primary tumor. $\times 10$.

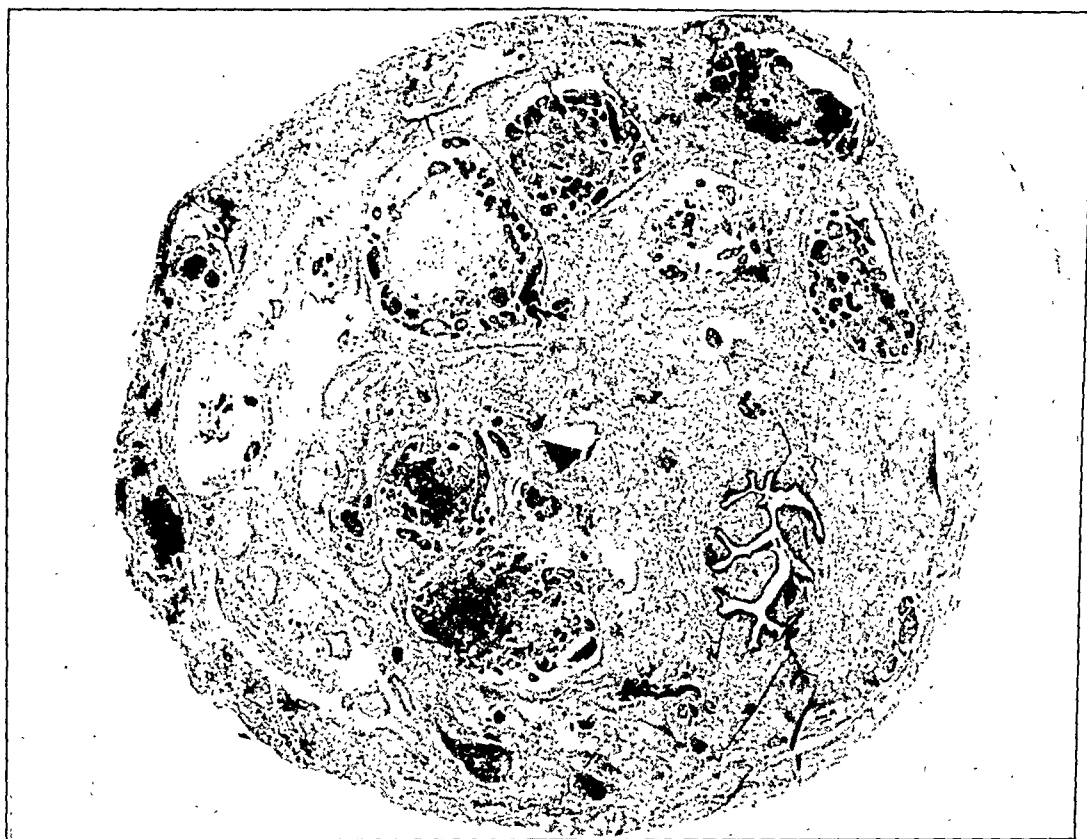
FIG. 23. Photomicrograph of a section of a dilated lymph vessel in the mesosalpinx. True emboli are present. In the center of the photomicrograph is an implantation of the foreign body type, such as often occurs in the implantation of ovarian carcinoma on the peritoneum. Compare with Fig. 21. $\times 54$.



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PLATE 10

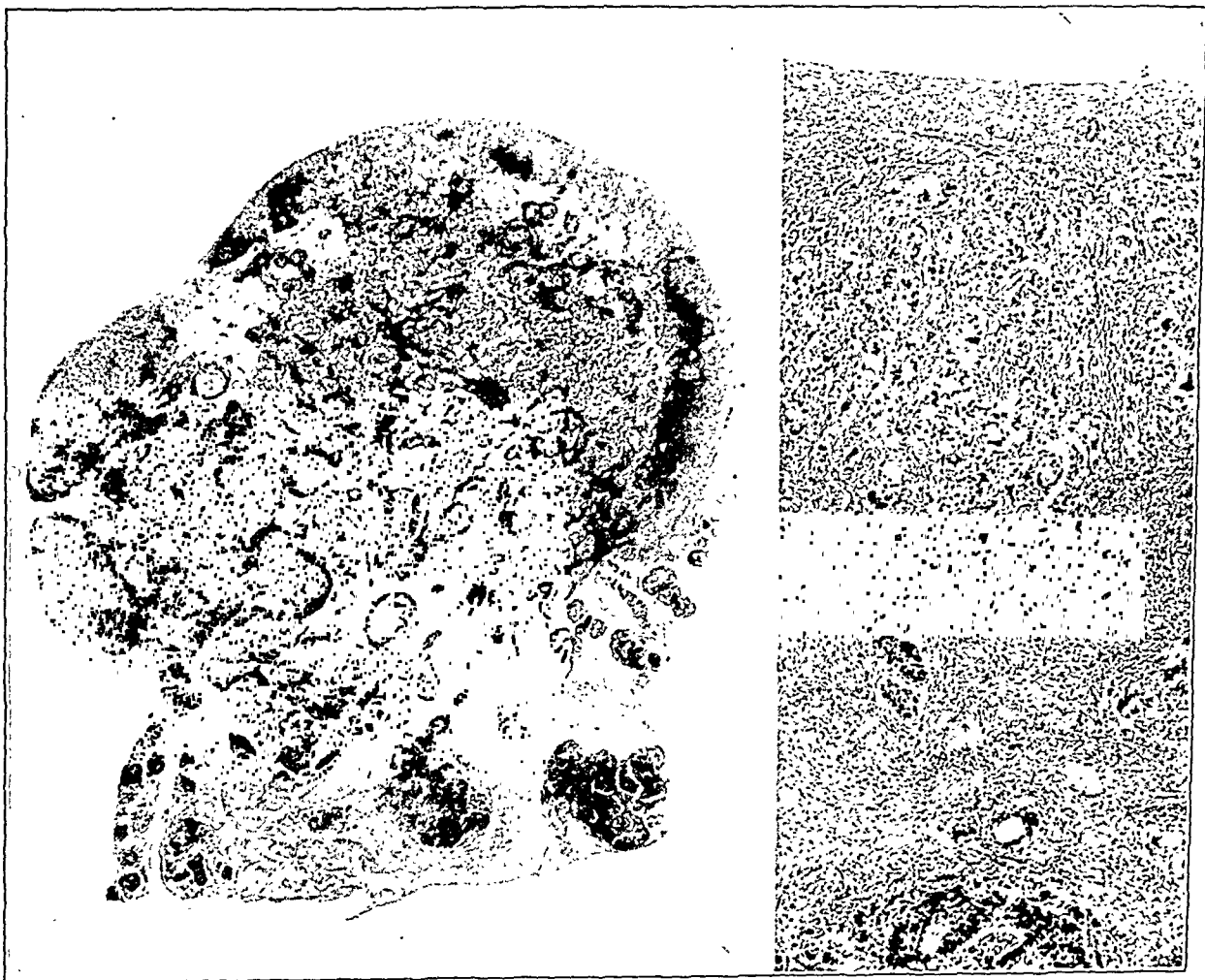
FIG. 24. Photomicrograph of a section of the tubal mucosa (Case 4) demonstrating the pathogenesis of carcinoma of this tube from carcinoma of the uterus, as a result of lymphatic permeation and metastasis (compare with Fig. 19). $\times 54$.

FIG. 25. Photomicrograph of a section of the ovary (Case 4), demonstrating the invasion of the ovary by carcinoma from that of the uterus through the lymphatics in the hilum of the ovary. $\times 5$.

FIG. 26. Photomicrograph of a section through the cortex of the ovary demonstrating the invasion of this portion of the ovary from carcinoma entering the ovary through its hilum and not its surface. $\times 54$.



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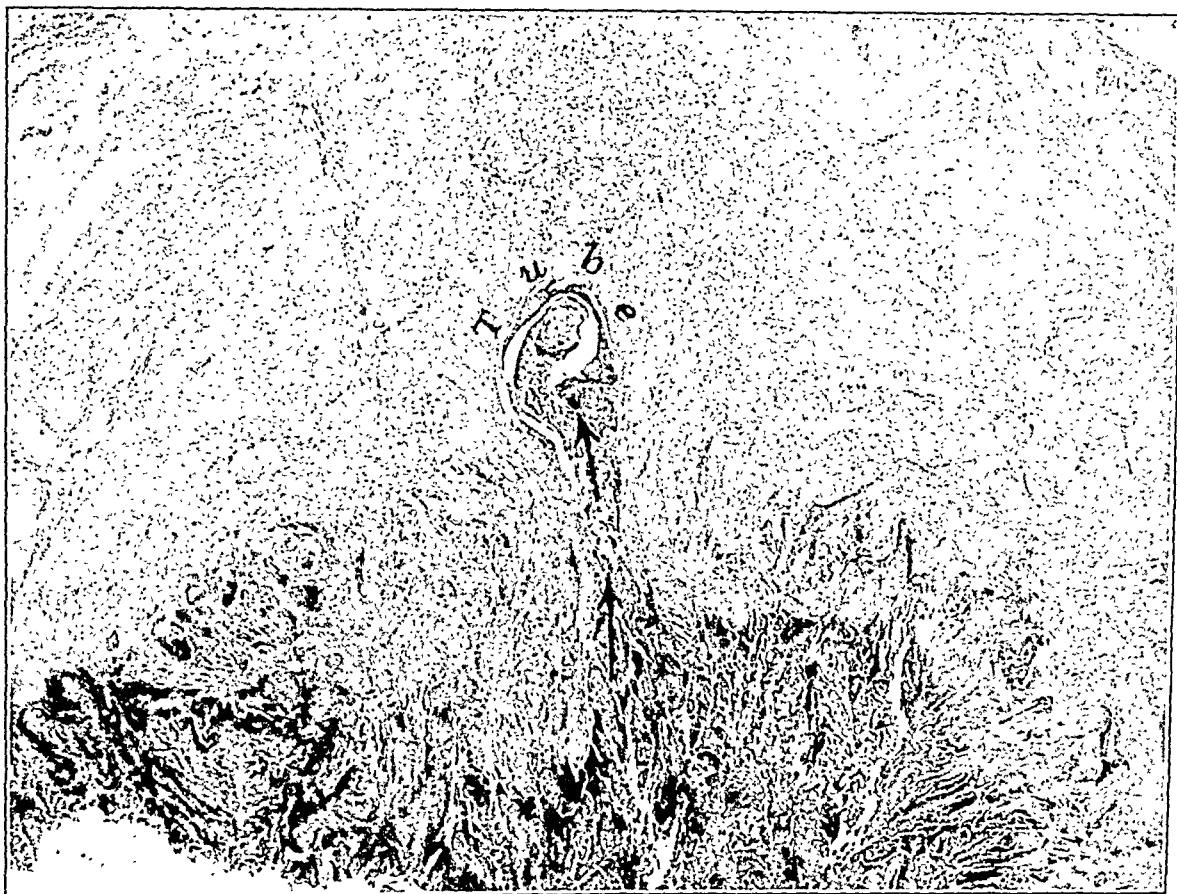


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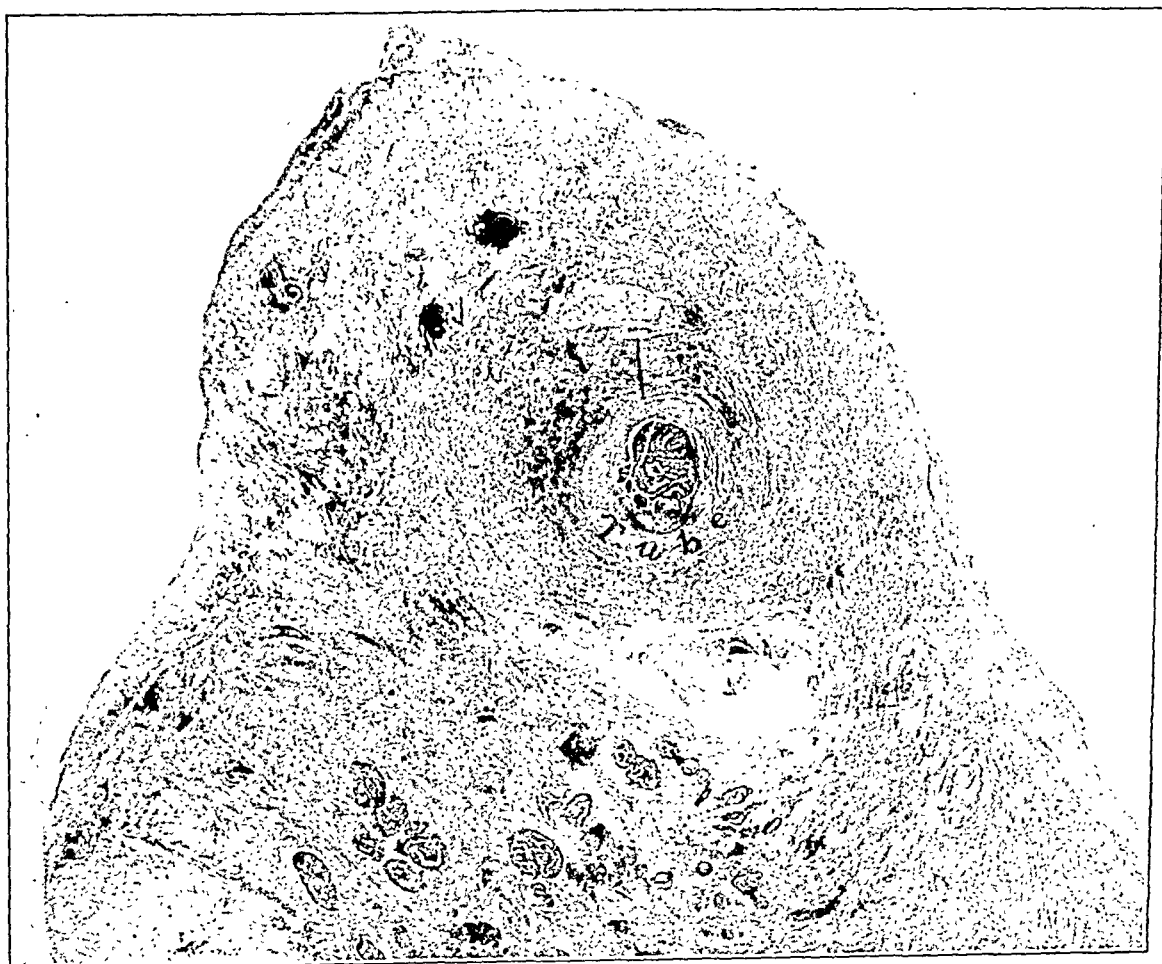
PLATE II

FIG. 27. Photomicrograph of a section (horizontal plane) of a portion of the uterine cornu including the uterine ostium of the tube from a patient aged 63 (Albany Hospital No. 8868-32), with adenocarcinoma of the body of the uterus involving the entire uterine mucosa. The patient had a previous operation 6 years before, when the pelvic floor was repaired, the appendix and both tubes and ovaries removed, and the uterus fixed to the abdominal wall. By continuous extension the carcinoma has invaded the uterine wall; and by a similar process it has passed through the uterine ostium of the tube into the uterine portion of the tube, replacing the mucosa of the latter. One can realize that particles of carcinoma might have broken off from the growth in the tube and might have migrated into the lumen of the tube beyond, had the tube not been removed at a previous operation. A similar condition was present in the opposite uterine cornu. $\times 10$.

FIG. 28. Photomicrograph of a section (vertical plane) of the uterine cornu, including the uterine portion of the tube from a patient aged 59 (Albany Hospital No. 8521-30), with an advanced adenocarcinoma of the body of the uterus. By continuous extension the carcinoma has invaded the uterine wall, penetrated the lymphatics, and also extended through the uterine ostium of the tube into the uterine portion of the latter. A similar condition was present in the opposite uterine cornu. Bilateral hydrosalpinx, evidently due to a previous pelvic infection was present. Both tubes were occluded in their uterine portion. $\times 10$.



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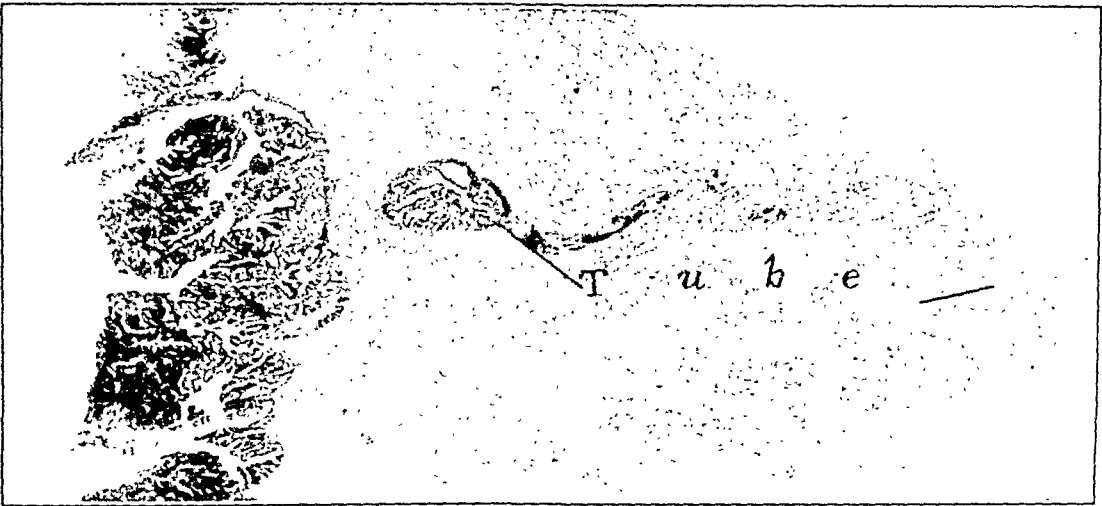
FIG. 29. Photomicrograph of a longitudinal section (vertical plane) of the left uterine cornu, including the uterine portion of the tube and a part of its isthmus, from a patient aged 56 (Albany Hospital No. 62-33), with a very early adenocarcinoma of the body of the uterus confined to the mucosa of the left uterine cornu. By continuous extension (shown by serial sections) the carcinoma has extended through the uterine ostium of the tube into the uterine portion of the latter, just as it spreads by replacing the mucosa of the body of the uterus. Carcinoma was not found in the tube beyond the place indicated in the photomicrograph. $\times 5$.

FIGS. 30, 31 and 32. Photomicrograph from a series of cross-sections of the uterine cornu and isthmus of the tube from a patient aged 64 (Albany Hospital No. 7587-32), with an advanced adenocarcinoma of the body of the uterus replacing the entire endometrium and invading the uterine wall. By continuous extension the carcinoma has passed through the uterine ostium of the tube into the uterine portion of the latter (Figs. 30 and 31). A cancer embolus is present in the lumen of the isthmus (Fig. 32); this is not continuous with the growth shown in the preceding photomicrograph. $\times 10$.

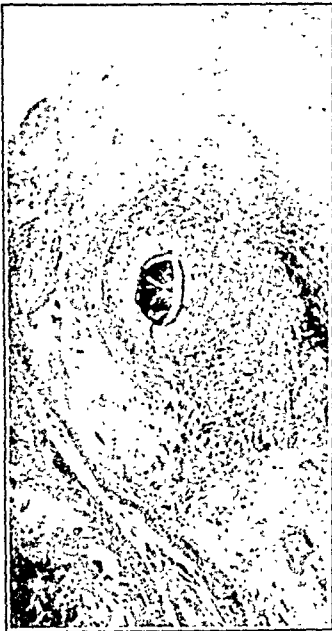
FIG. 33. Photomicrograph (higher magnification) of the carcinoma shown in Fig. 30. Its histological structure suggests a primary adenocarcinoma of the tubal mucosa. In reality it represents a graft of the uterine carcinoma in the tubal mucosa from the continuous extension of the primary tumor through the uterine ostium of the tube. $\times 54$.

FIG. 34. Photomicrograph (higher magnification) of the carcinoma shown in Fig. 31. It appears to be lying free in the lumen of the tube. In reality it is continuous with the growth shown in the preceding photomicrograph. It represents the outgrowths of the latter into the lumen of the tube as carcinoma grows into the lumen of a vein or lymph vessel. It is conceivable that emboli might arise from such a process and be carried to other portions of the tube, or even escape through the fimbriated end into the peritoneal cavity. Such emboli were found in the isthmus (Fig. 32), and likewise in the ampulla of the tube (see insert "a" and also Fig. 35). The embolus shown in insert "a" was in the ampulla of the tube about 1.5 cm. from the abdominal ostium. While some of the cells stained as well as those in the growth from which it was apparently derived, others did not. Karyorrhexis of some of the nuclei is present. $\times 130$.

FIG. 35. Photomicrograph of a portion of a section of the ampulla of the same tube shown in the preceding illustration. Another cancer embolus is shown situated between the folds of the tubal mucosa. The cells in this embolus stained better than those shown in the embolus of insert "a" of Fig. 34. Circumstantial evidence suggests that these emboli were present in the tube prior to the operation. Many interesting questions arise. Do these emboli wandering about in the lumen of the tube increase in size? Compare them with the smaller outgrowths shown in Fig. 34. How long will the cancer cells live in these emboli? Can they become grafted in the tubal mucosa if a suitable soil is found or created? See Fig. 42. What might happen if they escaped into the peritoneal cavity? $\times 130$.



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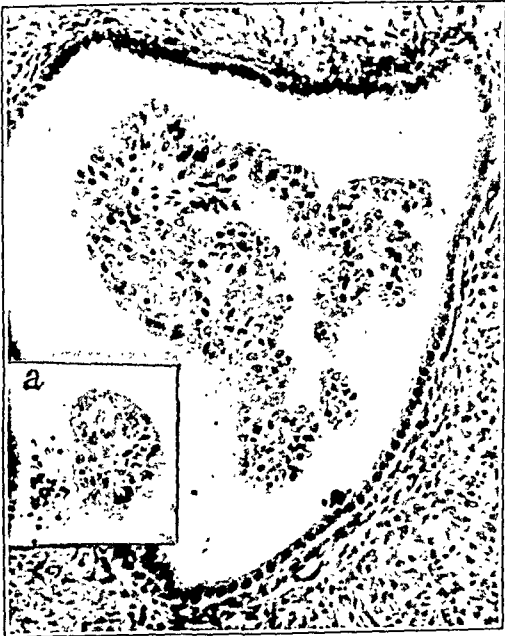
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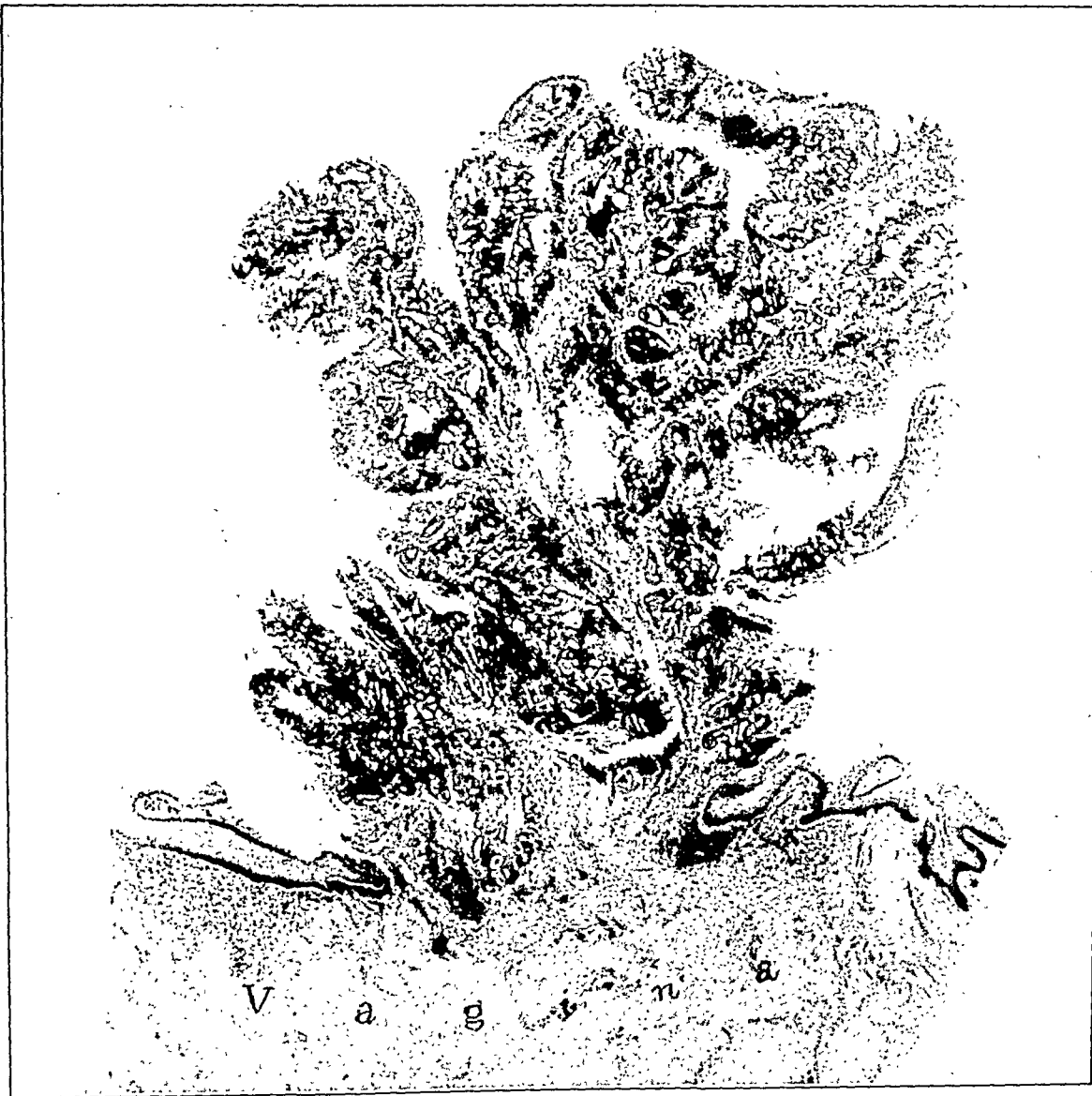


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PLATE 13

FIG. 36. Photomicrograph of a section of a metastasis of a papillary adenocarcinoma of the uterus to the vagina (Case 5). Circumstantial evidence indicates that it is a wound implantation. The hymen was injured in exposing the cervix while obtaining a biopsy prior to the introduction of radium 2 months before. The papillary carcinoma, with the same histological structure as the primary tumor, has arisen in the scar of this wound. Note the superficial character of the tumor and the normal stratified epithelium on both sides of its base. Compare also with Fig. 21, demonstrating a similar papillary carcinoma implanted on the surface of a lymph vessel. The various stages in the development of the implants in the two situations are the same, namely, the grafting of cancer cells on or in a wound with the attempted healing of the wound and the continued growth of the cancer cells in this situation. This was the only vaginal metastasis found. $\times 15$.

FIG. 37. Photomicrograph of a possible implantation carcinoma in the uterine mucosa secondary to adenocarcinoma of the uterus from a patient aged 57 (Albany Hospital No. 5221-29). On incising the uterus, after its removal, this papillary growth was noted near the primary tumor but grossly not continuous with the latter. Serial sections failed to demonstrate any continuity between the primary uterine tumor and the small papillary growth near it. Although a multicentric origin cannot be excluded, the possibility of an implantation metastasis must be considered. Since cancer cells can become implanted on endothelial surfaces and in vaginal wounds why not in uterine mucosa, if a suitable soil be created? $\times 8$.



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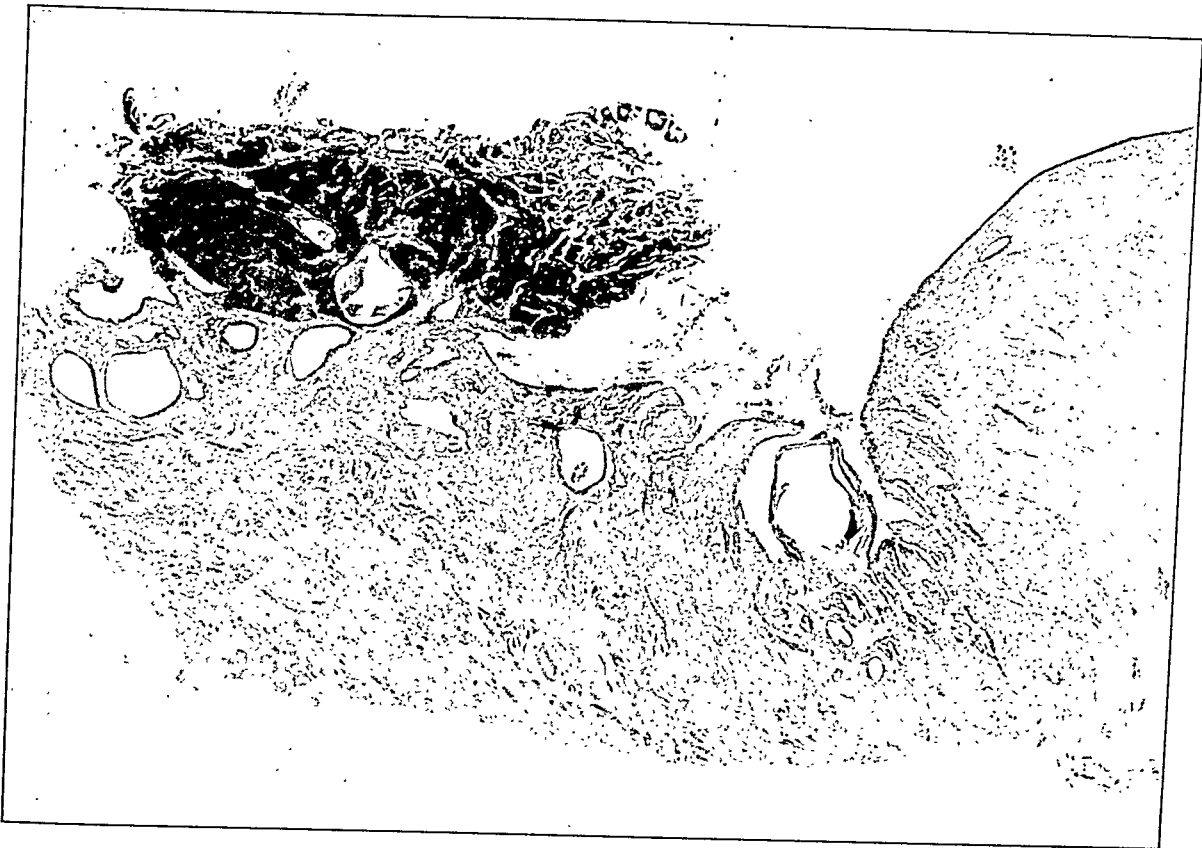
PLATE 14

FIG. 38. Photomicrograph of a section of a metastatic carcinoma of the anterior vaginal wall, secondary to an advanced carcinoma of the uterus (Case 6). The carcinoma was situated in the anterior vaginal wall beneath the urethra and was partially exposed. It is impossible to state the method of its origin, whether by implantation or retrograde metastasis through the lymph vessels or veins. The patient had a diagnostic curettage a year ago and repeated X-ray treatments since then in another hospital. Compare with Fig. 113. $\times 10$.

FIG. 39. Photomicrograph of a metastatic carcinoma of the cervical mucosa secondary to the carcinoma of the uterus (Case 6). The growth is situated on the mucosa of the posterior wall of the cervix and is not continuous with the primary tumor. The very superficial character of the growth, the absence of any lymphatic involvement and the history of a curettage a year ago all point to an implantation in a wound of the cervical mucosa. Compare with the conditions shown in Figs. 21, 36 and 37. $\times 10$.



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FIG. 40. Photomicrograph of a longitudinal section of the distal end of the left tube (Case 6). Note the superficial character of the carcinoma and the infolding of the tubal fimbriae as a result of their reaction to the tumor. In time the fimbriated end of the tube would have become occluded. Many sections of this block were studied and it was shown that what appear as multiple tumors in this photomicrograph are in reality all one growth. Is this carcinoma an instance of multicentric origin, or is it secondary to the uterine tumor by metastasis through the lymphatics or veins? Carcinoma was not found in the lymphatics of the tube. However, clumps of judged cancer cells were found in the veins of the peripheral portion of the wall of the uterus and also in one vein in the tubal wall near the uterus. Can an implantation of cancer cells escaping through the lumen of the tube be excluded? $\times 10$.

FIG. 41. Photomicrograph (higher magnification) of a portion of the section shown in the preceding illustration. The general histological structure of the growth is that of one arising from the tubal epithelium, or of cancer cells grafted in the tubal mucosa. Other sections demonstrated that the portion of the carcinoma marked "b" was continuous with that on the surface, just as the portion marked "c" is continuous with the latter. Note the cellular débris, containing clumps of cancer cells, in the lumen of the tube. It is conceivable that some of this material might have escaped into the peritoneal cavity if it had not been prevented by the infolding and agglutination of the tubal fimbriae. It is also conceivable that it might have migrated toward the uterus. See next illustration. $\times 54$.

FIG. 42. Photomicrograph of a portion of a cross-section of the ampulla of the tube proximal to the block from which the preceding section was made. Débris at "a" and "b," similar to that shown in the preceding illustration, is present in the lumen of the tube. A clump of cancer cells "a," similar to those marked "a" of the preceding illustration, is attached to the tubal mucosa in a cleft between two mucosal folds — a situation most favorable for splinting a graft (compare with Fig. 35). The epithelium beneath this clump has disappeared — the only area in the entire cross-section of the tube where the tubal epithelium is lacking. At this point there appears to be an intermingling of the cells of the tube with those of the clump. Many sections were made of this block and showed that clump "a" was not continuous with carcinoma elsewhere in the tube. Could the phenomenon shown here be interpreted as the grafting or implantation of cancer cells in tubal mucosa? See next illustration. $\times 130$.

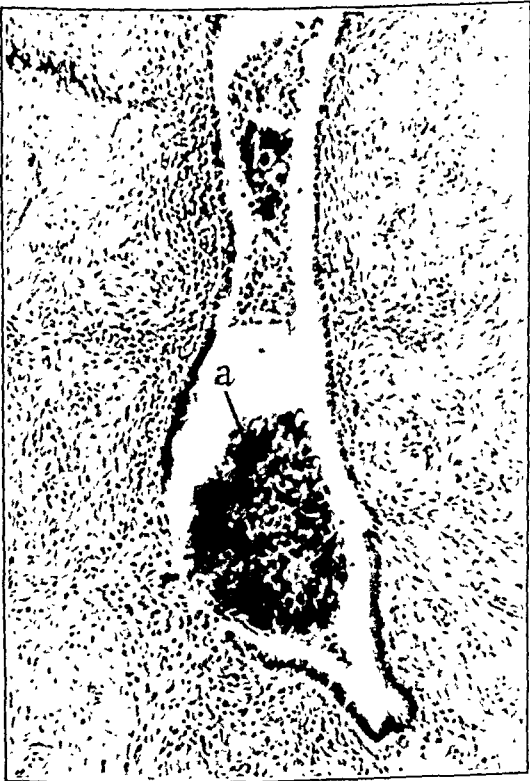
FIG. 43. Photomicrograph of a portion of the mesosalpinx of the isthmus of the tube shown in Fig. 28 demonstrating clumps of cancer cells in a lymph vessel. Many sections, some in series, demonstrated that the carcinoma in this situation was not continuous with carcinoma elsewhere in the specimen. Implantation is the only rational interpretation of the pathogenesis of this condition, which arose from the grafting of cancer cells, which had escaped into the lymphatic from the primary uterine carcinoma, on the endothelial lining of this lymph vessel. If so, could not the phenomenon in the preceding illustration well represent the grafting of cancer emboli in tubal mucosa? The fundamental stages of the development of the lesions in the two situations were the same, as is also their histological structure. $\times 130$.



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Carcinoma of Tubes and Ovaries

PLATE 16

FIG. 44. Photomicrograph of a section of the distal end of the left tube and a portion of the ovary adherent to it (Case 7). The tube was distended with blood-tinged fluid. Scattered throughout the tubal mucosa are multiple carcinomas of various sizes, thus suggesting different ages. Four of these are shown in this photomicrograph, "a," "b," "c" and "d." The largest of these is indicated by the letter "a." Other sections demonstrated that it was attached to the tubal mucosa by a slender pedicle. Carcinoma was not found in the lymphatics or veins in any portion of the specimen. I believe that the pathogenesis of these lesions admits of only two interpretations: first that of a multicentric origin for the carcinomas in the uterus and tube, all caused by the same agent or agents acting at different times; secondly, that of implantation, cancer cells escaping from the uterine growth into the lumen of the tube and becoming grafted in the tubal mucosa with subsequent grafts from this source. $\times 10$.



PLATE 17

- FIG. 45. Photomicrograph (higher magnification) of carcinoma "b" shown in the preceding illustration. There is nothing in this section to indicate the origin of the carcinoma, whether from a differentiation of tubal epithelium, or from grafted epithelium. I believe that the relation between the malignant and the normal epithelium, as seen in the advancing edge of the growth, would be the same in either instance. The condition shown here suggests that the carcinoma might have started in a cleft between two folds of the tubal mucosa. This would be an ideal situation for the retention of a clump of cancer cells. Compare with Figs. 35 and 42. $\times 130$.
- FIG. 46. Photomicrograph of carcinoma "d" shown in Fig. 44. Note the superficial character of the growth. It has the same histological structure as that of the uterine tumor shown in the next illustration. $\times 25$.
- FIG. 47. Photomicrograph of a section of a portion of the uterine cornu including the uterine mucosa replaced by carcinoma, and the uterine portion of the tube. Unfortunately the uterine ostium of the tube was missed in trimming the block. Nevertheless, carcinoma in this situation might have extended into the uterine ostium of the tube and particles from the growing papillary outgrowths (twigs) of the carcinoma in this situation might have escaped into the lumen of the tube, just as similar fragments escape into the lumen of a lymphatic from the ends of rootlets which have penetrated these vessels. The grafting of some of the particles in the tubal mucosa could account for the secondary carcinoma of the tube. $\times 10$.



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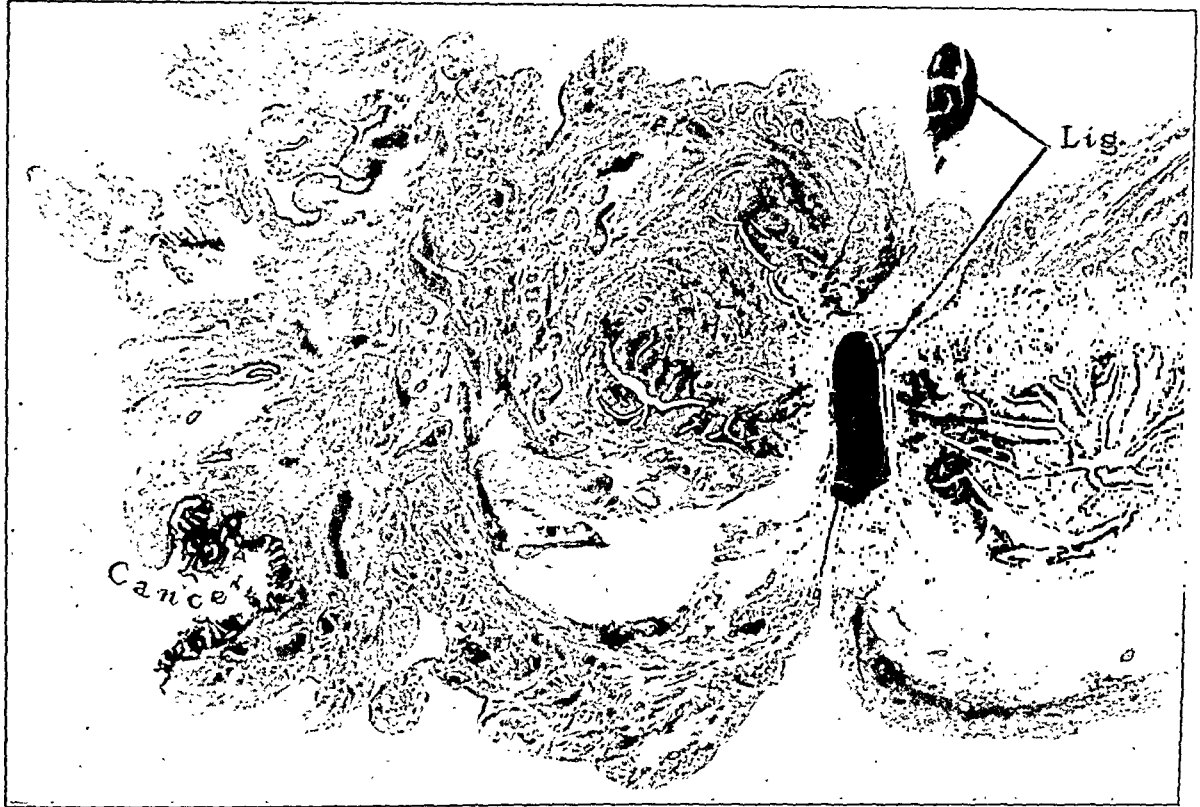
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PLATE 18

- FIG. 48. Photomicrograph of a sagittal section of the distal end of the left tube (Case 8). The uterus contained multiple myomas and an advanced adenocarcinoma of its body. Aside from peritoneal adhesions about the fimbriated end of this tube and the ovary they, as well as the opposite tube and ovary, appeared normal. There was no gross evidence of peritoneal carcinomatosis. Many sections of this block were studied and showed that the judged carcinoma of the tubal fimbriae appeared superficial in all. Carcinoma was not found in the tubal wall beneath it or in any other portion of the tube. From the histological structure of the growth as well as from circumstantial evidence, it must have arisen either from a differentiation of the tubal epithelium, or the grafting of cancer cells escaping from the uterine growth through the lumen of the tube. The latter seems more logical to me. The tube was ligated ("lig.") proximal to the fimbriated end at the operation. This would not have been done had the carcinoma of the fimbriae been recognized. $\times 10$.
- FIG. 49. Photomicrograph (higher magnification) of the carcinoma shown in the preceding illustration showing better the character of the lesion. Is it a true carcinoma or merely a hyperplasia of the tubal epithelium? See next illustration. $\times 54$.
- FIG. 50. Photomicrograph of the papillary adenocarcinoma of the uterus. There is such a marked similarity between this and the condition of the tubal fimbriae that I believe they are the same. $\times 54$.



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PLATE 19

FIG. 51. Photomicrograph of a section of the wall of a dilated tube with multiple superficial patches of adenocarcinoma in its lining. These areas are of various sizes, thus suggesting different ages. These lesions were associated with an adenocarcinoma of the body of the uterus, which had replaced the greater portion of the mucosa of the fundus, including that of both cornua (Case 9). Both tubes were dilated as in hydrosalpinx, with occlusion of their abdominal ostia and contained multiple carcinomas, as indicated in this photomicrograph. The multiple carcinomas of different sizes in the two tubes indicate either a multicentric origin, multiple metastases through the blood or lymph stream, or the implantation of particles of cancer which we realize must have been floating about in the lumen of the tube. Cancer emboli were not found in the veins or lymphatics of the tubal wall, but were present in the lumen of the tube. All the tubal carcinomas were superficial. Compare with the judged implantation carcinomas shown in Figs. 21, 36 and 39. $\times 10$.

FIG. 52. Photomicrograph (higher magnification) of area "a" of the preceding illustration. Note the very superficial character of the growth, that it has replaced the tubal mucosa at the right and has arched over the tubal epithelium to the left to become attached again to the tubal wall. $\times 54$.

FIG. 53. Photomicrograph (higher magnification) of area "b" of Fig. 51, emphasizing the superficial character of the growth in this situation and creating the impression that it has been added to the surface of the lining of the tube. $\times 54$.



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PLATE 20

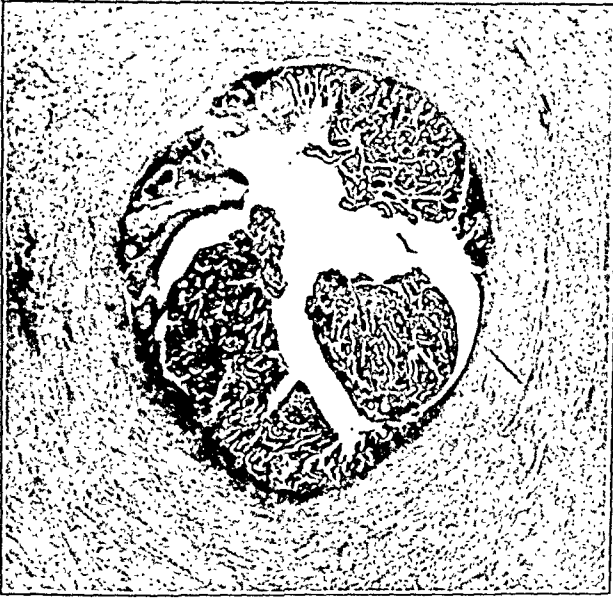
FIG. 54. Photomicrograph of one of a series of sections of the uterine cornu and uterine portion of the tube (Case 9). By continuous extension the carcinoma of the uterus has invaded the uterine portion of the tube through its uterine ostium. Note the superficial character of the growth of the same histological structure as that shown in Fig. 51, and the way it has replaced the tubal mucosa. $\times 10$.

FIG. 55. Photomicrograph of a section of the uterine portion of the tube from the same series shown in Fig. 54, but distal to the latter. The carcinoma in this section is continuous with that shown in the preceding one and has occluded the lumen of the tube (compare with Fig. 30). It is attached to the tubal wall in only one area. It is conceivable that particles of cancer from such a growth might as readily escape into the lumen of the tube beyond it, as cancer emboli escape from a similar growth that has penetrated the lumen of a vessel. See next illustration. In both instances we are dealing with the advancing (invading and therefore growing) part of the tumor. $\times 10$.

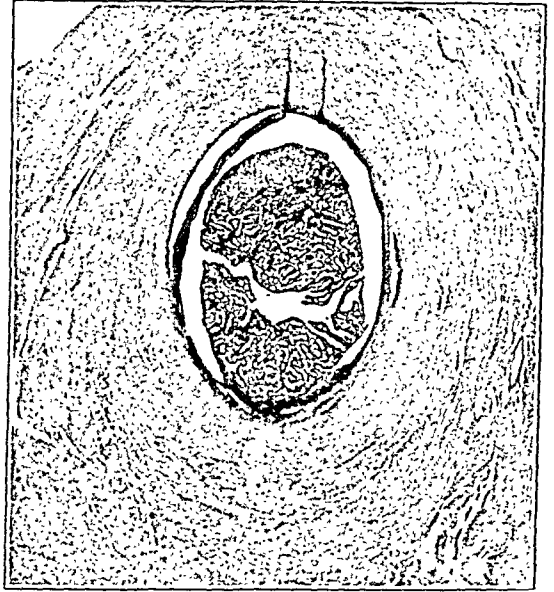
FIG. 56. Photomicrograph of a section of the uterine portion of the tube distal to the section shown in the preceding illustration. Note the carcinoma in the lumen of the tube. Serial sections demonstrated that some of this was continuous with the growth shown in the preceding illustration. Free particles of the growth, however, are present, which, with the occlusion of the lumen of the tube by the carcinoma shown in Fig. 55, might migrate in the tube towards its fimbriated end and even escape into the peritoneal cavity if the abdominal ostium were patent. $\times 54$.

FIG. 57. Photomicrograph of a portion of a cross-section of the isthmus of the tube shown in the preceding illustration. The lumen of the tube is becoming occluded by an unusual reaction. See next illustration. $\times 54$.

FIG. 58. Photomicrograph (higher magnification) of a part of the section shown in the preceding illustration. I do not know how to interpret the condition shown here. I am tempted to believe that it must be a reaction arising from something escaping into the lumen of the tube from the growth in the uterus, or that in the tube. Are the large epithelial cells, to the left, atypical cancer cells or hypertrophied and distorted tubal epithelium? What is the relation between the carcinoma of the uterus and that in the two tubes? Are all the isolated carcinomas in the three situations tumors of multicentric origin due to the same agent, or agents acting at different times? The escape of cancer cells from the uterine growth into the lumina of the tubes, with subsequent implantation on the lining of the tubes followed by secondary implantations, would account for the condition present in these three situations. $\times 130$.



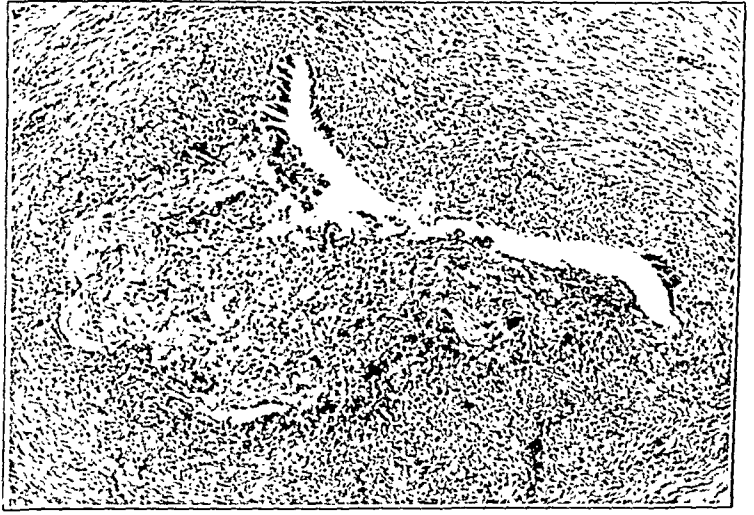
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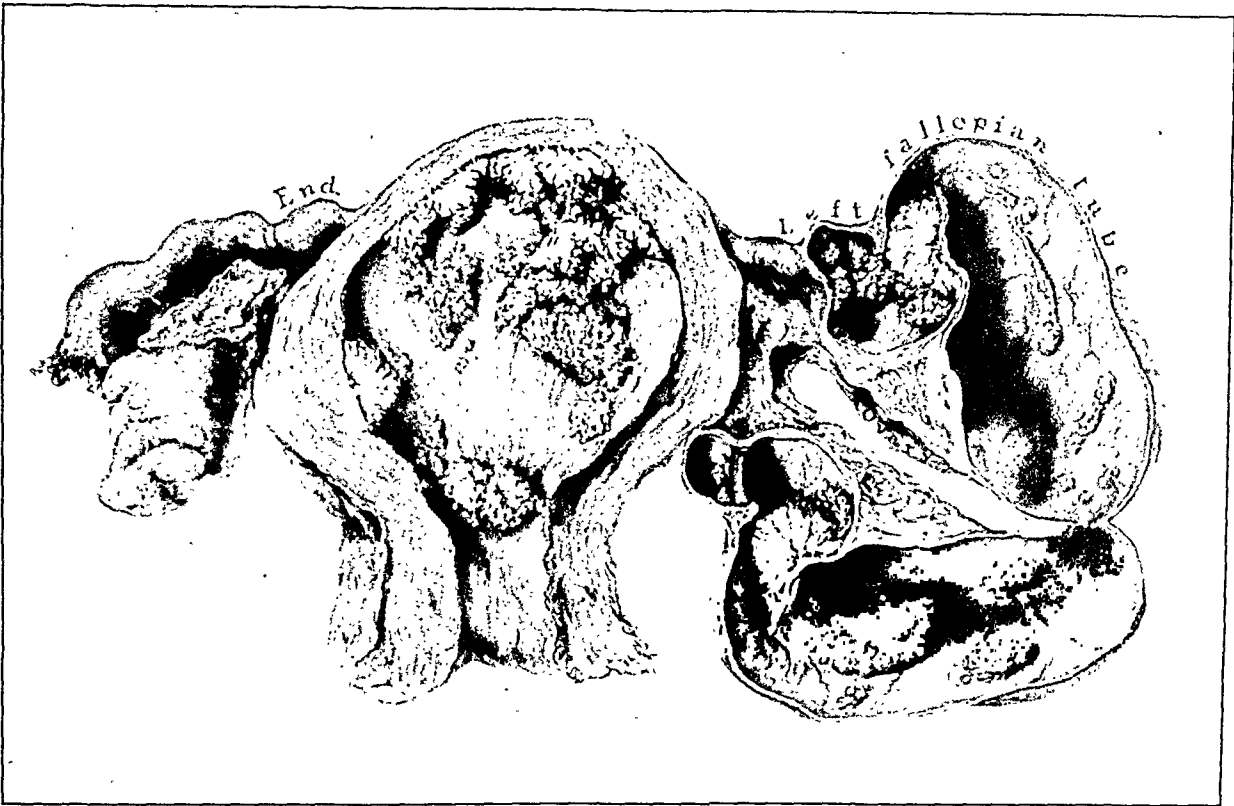


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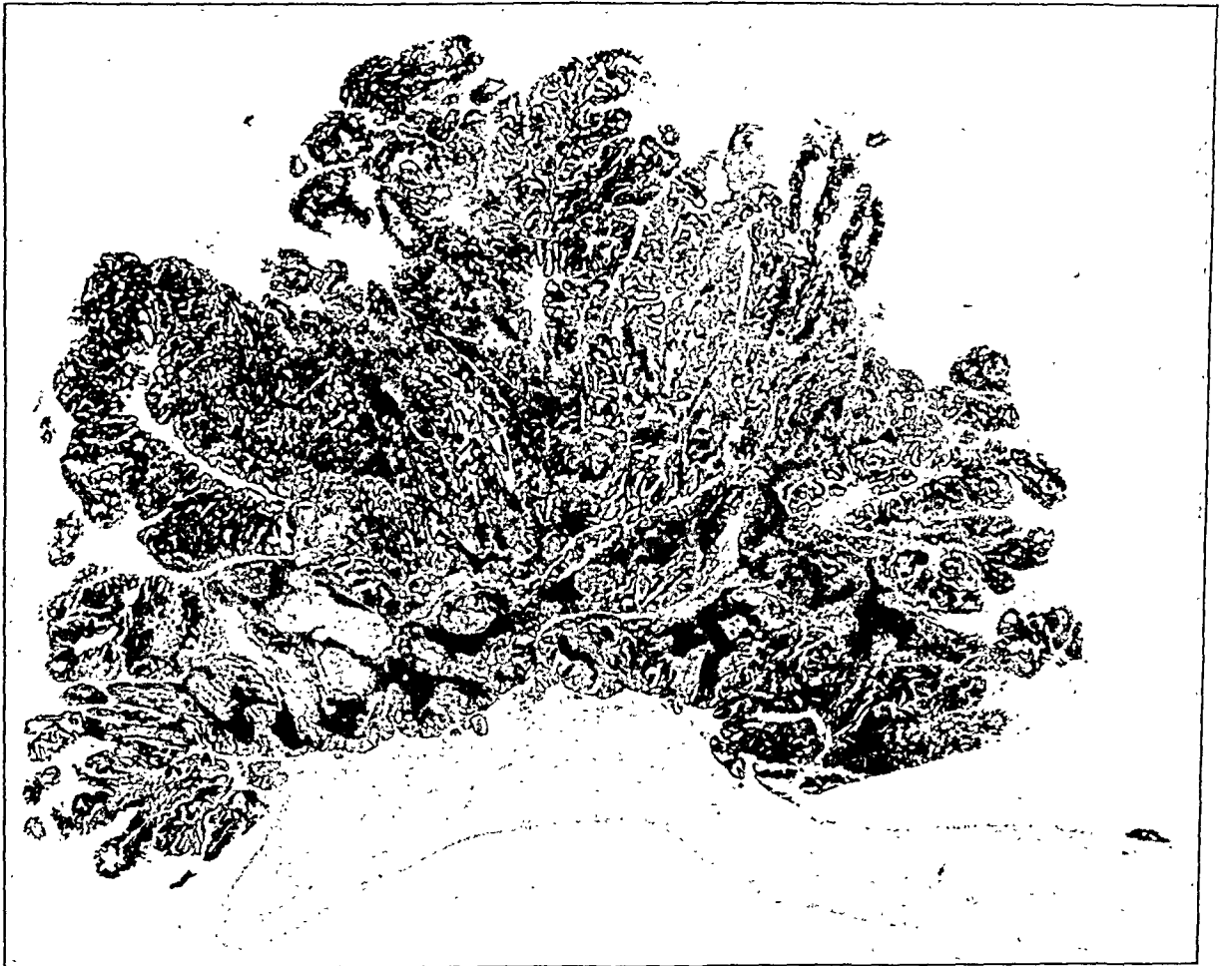
PLATE 21

FIG. 59. Papillary adenocarcinoma of the body of the uterus and of the left tube (Case 10). Multiple carcinomas of various sizes, therefore suggesting different ages, are present in both the uterus and the tube. Is this an example of the multicentric origin of carcinoma due to the same agent or agencies acting at different times, causing a like differentiation of uterine and tubal epithelium into carcinoma? Is it possible that the growth in both the uterus and tube may have spread by the implantation of cancer cells escaping into these cavities? What is the relation between the carcinoma of the uterus and that of the tube? Could one be secondary to the other? Why is carcinoma present in the left tube and not in the right? See Figs. 61 to 68, inclusive. $\times 2/3$.

FIG. 60. Photomicrograph of a section of the largest growth present in the left tube. Similar and even larger growths were present in the uterus. Note the superficial character of the tumor. Many sections (some in series) were made of this block and it was demonstrated that the carcinoma had spread from the base of this papilloma, replacing the tubal epithelium. On inspecting the surface of this papilloma, one can readily understand how clumps of living cancer cells might break off from the growing tips of the papillary outgrowths and be disseminated into the cavity of the tube, just as cells escape into the peritoneal cavity from a similar growth on the surface of the ovary or as carcinoma escapes into the lumen of a lymph vessel. The small isolated growths in the tube are not unlike implantations on the peritoneum and in lymph vessels. $\times 10$.



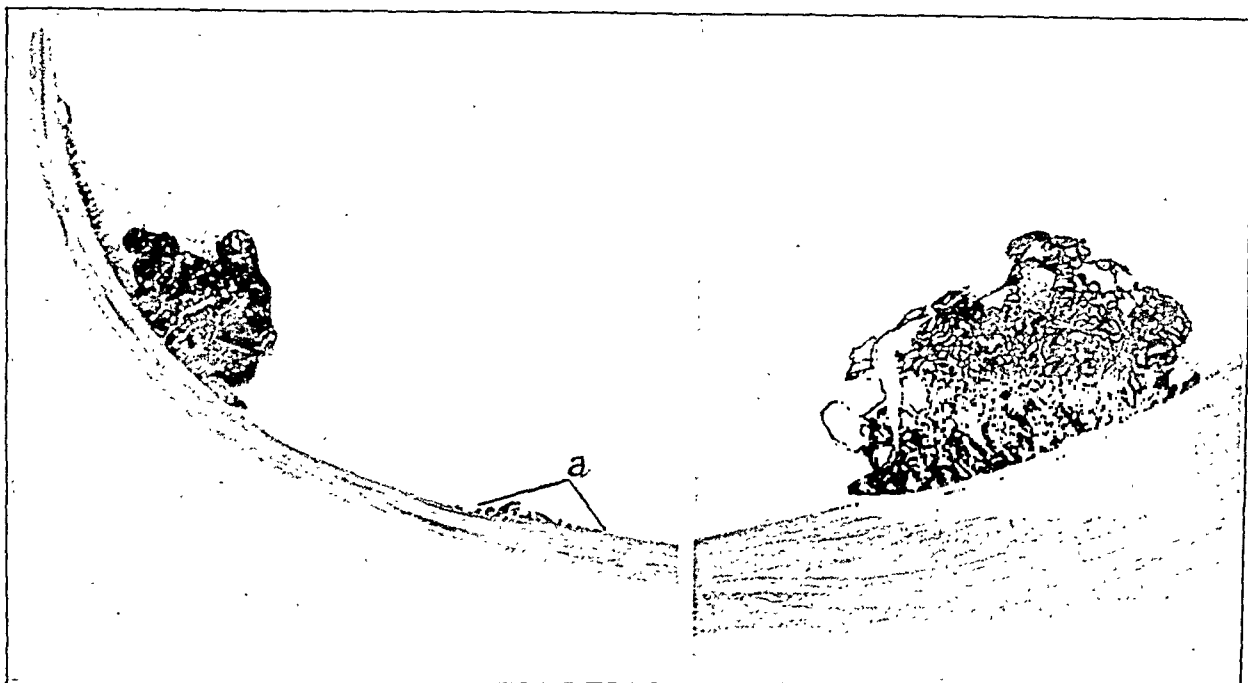
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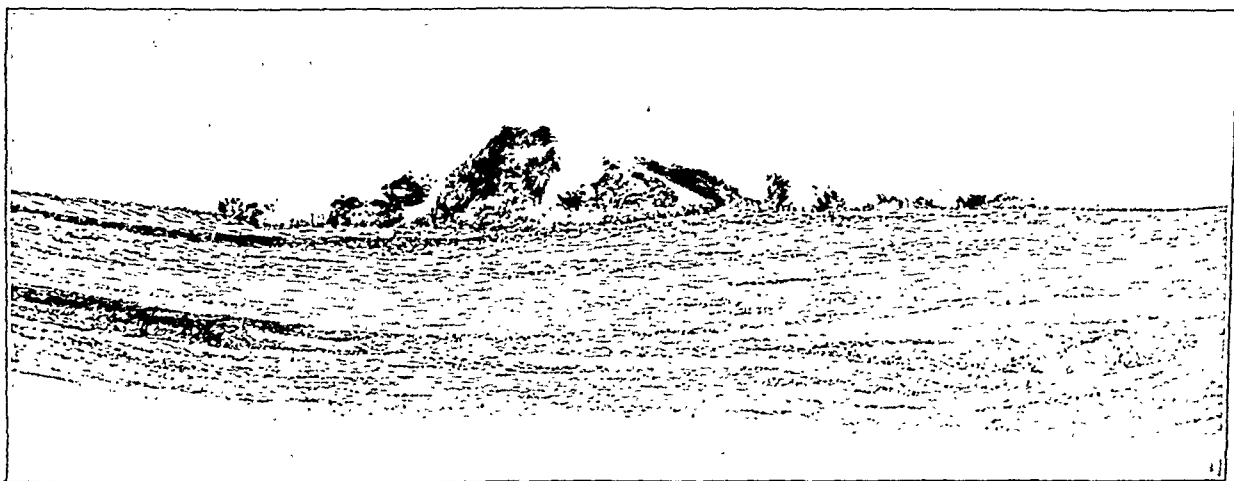
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PLATE 22

- FIG. 61. Photomicrographs of two sections of the tubal wall showing three discrete patches of carcinoma replacing the tubal mucosa. The variation in their sizes suggests different ages. Note that the one to the left is spreading over and replacing the tubal mucosa. Compare these with the tumors shown in the preceding illustration, all the same magnification. $\times 10$.
- FIG. 62. Photomicrograph (higher magnification) of the patch of cancer "a" of the preceding illustration. I believe that the cancer began in the center of the patch where the growth is the most advanced and probably spread in all directions. $\times 54$.
- FIG. 63. Photomicrograph (still higher magnification) of the advancing edge of the patch of carcinoma shown in the preceding illustrations. One can see that the growth has replaced the tubal epithelium and is advancing over it to the right. $\times 130$.



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PLATE 23

FIG. 64. Photomicrograph of a portion of the uterus showing some of the smaller patches of carcinoma indicated in Fig. 59. Note the very superficial character of the growth and the way it has apparently spread over the mucosa by direct extension and also, I believe, by implantation. $\times 10$.

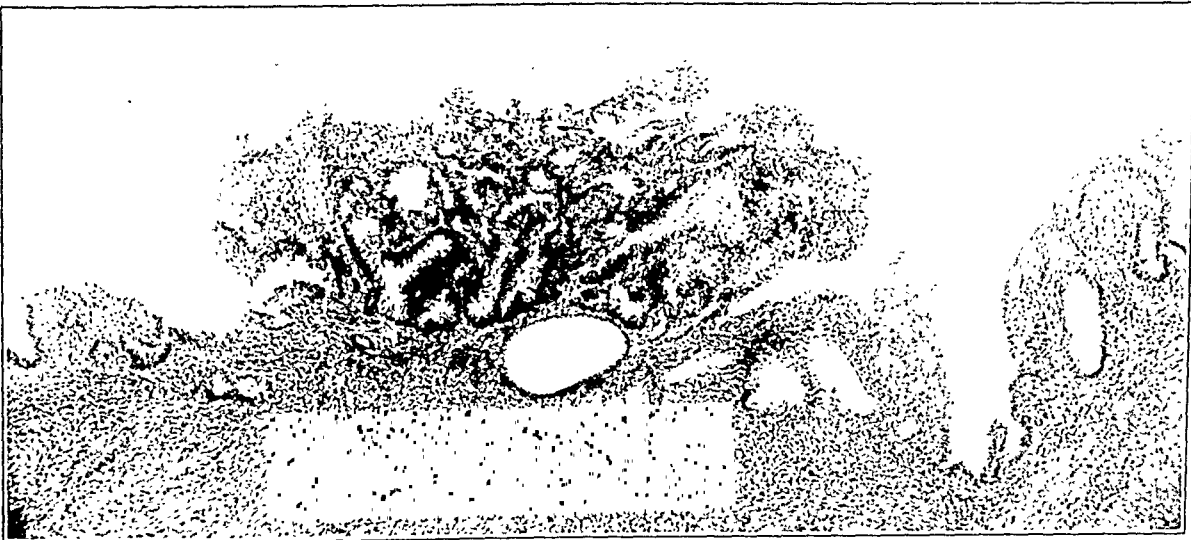
FIG. 65. Photomicrograph (higher magnification) of patch "a" of the preceding illustration. Many sections of this block demonstrated that this patch of carcinoma was not continuous with that elsewhere in the specimen. Note its very superficial character, as though added to the surface of the uterine mucosa. Its pathogenesis cannot definitely be determined. However, one is impressed that in some way it must be derived from the carcinoma of the uterus near it. It is not continuous with the latter. Compare with Figs. 21, 36, 39 and 51. $\times 54$.

FIG. 66. Photomicrograph of a section of the left cornu of the uterus, including the uterine ostium of the tube. By serial sections one could follow the carcinoma, replacing the uterine mucosa, into the ostium of the tube, thus plugging the latter, as in Figs. 30 and 55. $\times 10$.

FIG. 67. Photomicrograph of a section of the uterine ostium of the tube, distal to the preceding section, showing carcinoma in its lumen continuous with that shown in the preceding illustration. Clumps of cancer cells from such an area might easily escape into the patent tube or even into the peritoneal cavity, should the fimbriated end be open. A study of this specimen indicates that implantation of carcinoma might have occurred in both the uterine and tubal mucosae. The last two illustrations indicate a way that cancer cells from the uterine tumor might have escaped into the lumen of the tube. The isthmus of the tube was patent. $\times 25$.



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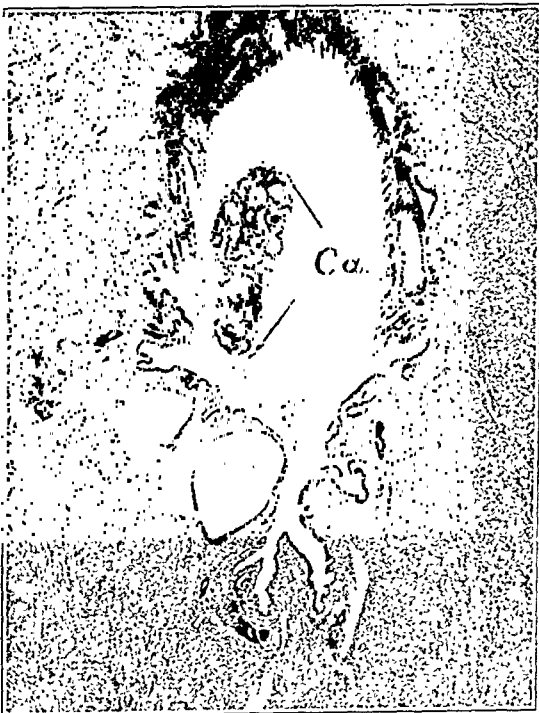


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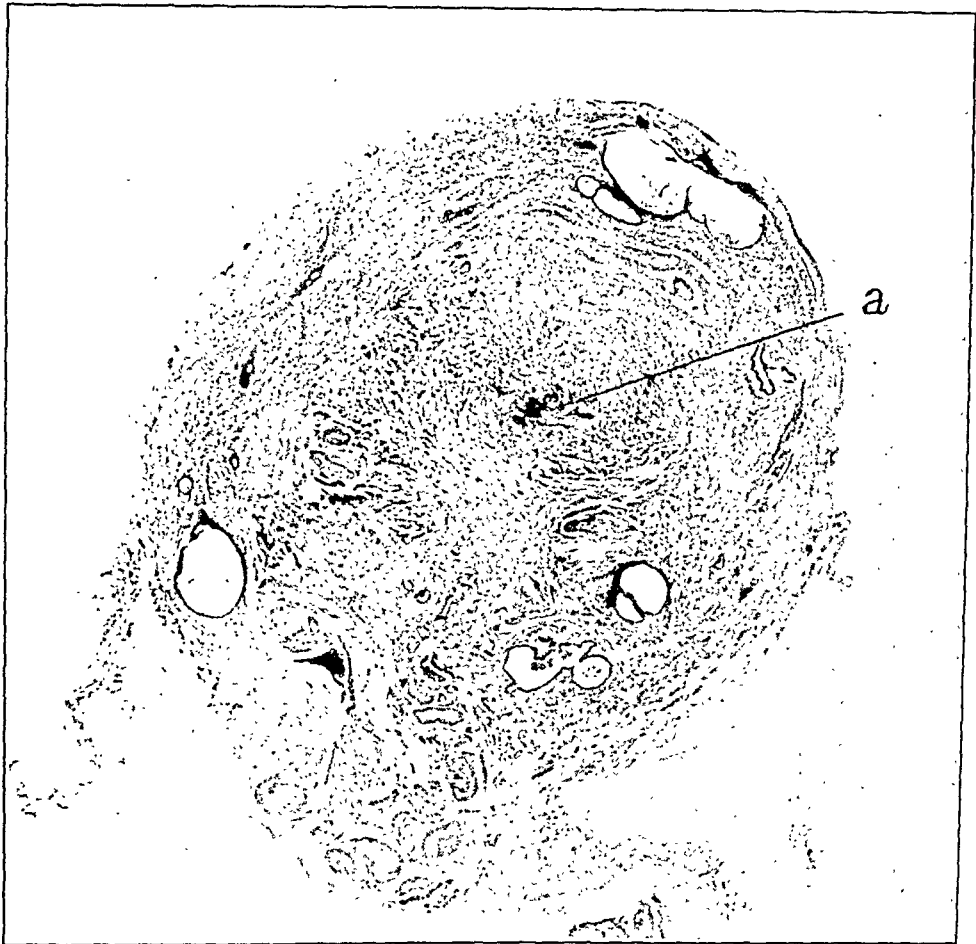
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Carcinoma of Tubes and Ovaries

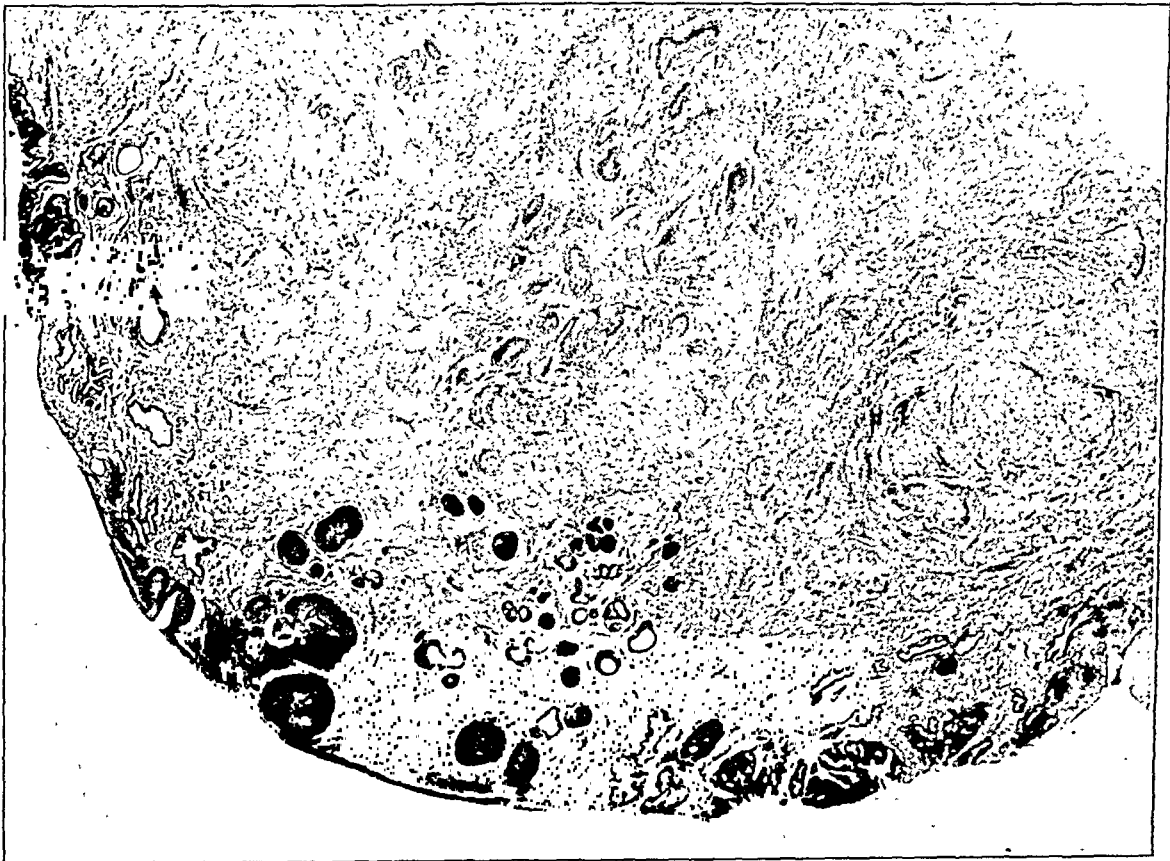
PLATE 24

FIG. 68. Photomicrograph of a cross-section of the isthmus of the right tube. Carcinoma was not found in the right tube. An endosalpingeosis (see "End." of Fig. 59) is present in this tube with occlusion "a" of its lumen. This probably was present before the growth developed in the uterine mucosa, and would prevent the escape of particles of the uterine growth into the lumen of this tube, as could have occurred in the opposite tube. $\times 10$.

FIG. 69. Photomicrograph of a section of the cervix (Case 10), showing early squamous cell carcinoma "c" arising from the mucosa of the vaginal portion of the cervix near its junction with the mucosa of the cervical canal. This is the strongest argument in the case favoring the multicentric theory for the origin of the many carcinomas of different sizes in the uterus and left tube. Even so it does not prove that these carcinomas are not of implantation origin. $\times 10$.



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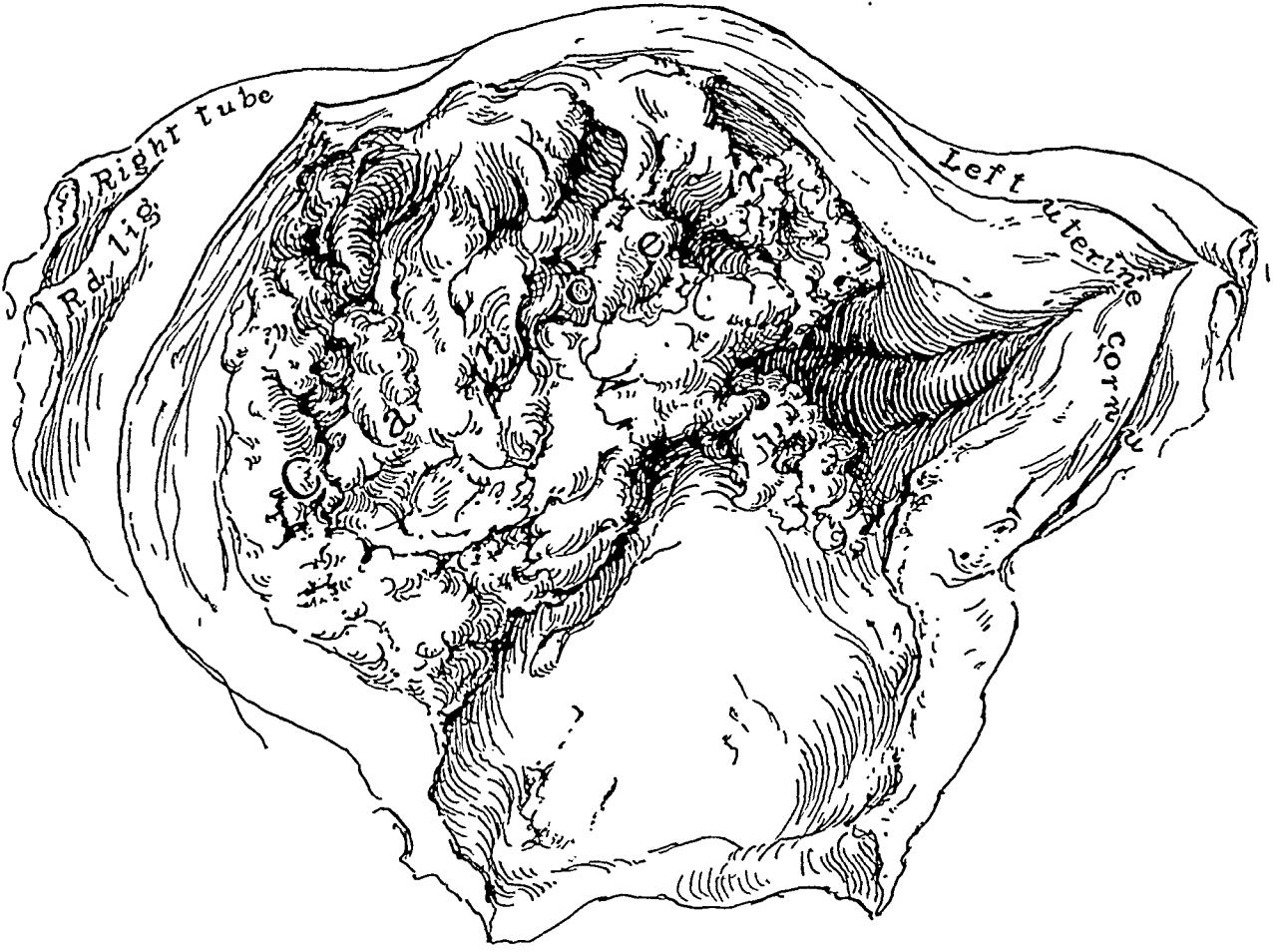
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PLATE 25

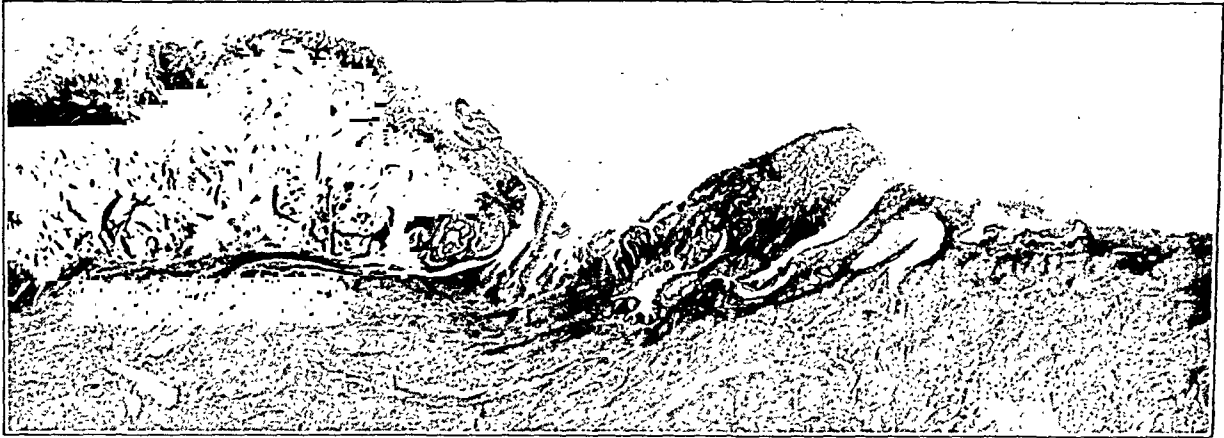
FIG. 70. Adenocarcinoma arising in the right cornu of a markedly bicornuate uterus (Case 11). Both tubes and ovaries had been removed with the uterus. Carcinoma was not found in either the left tube or ovary but was present in the right tube and ovary. Natural size.

FIG. 71. Photomicrograph of a section of the advancing margin of the cancer. Note the superficial character of the growth and the way it replaces and rides over the adjacent uterine mucosa. $\times 54$.

FIG. 72. Photomicrograph of a section of the uterine ostium of the right tube showing clumps of cancer cells in its lumen undoubtedly derived from that portion of the growth filling the right uterine cornu (see Fig. 70). Similar clumps of cells might easily have gone farther into the lumen of the tube and even out through the fimbriated ostium into the peritoneal cavity. $\times 54$.



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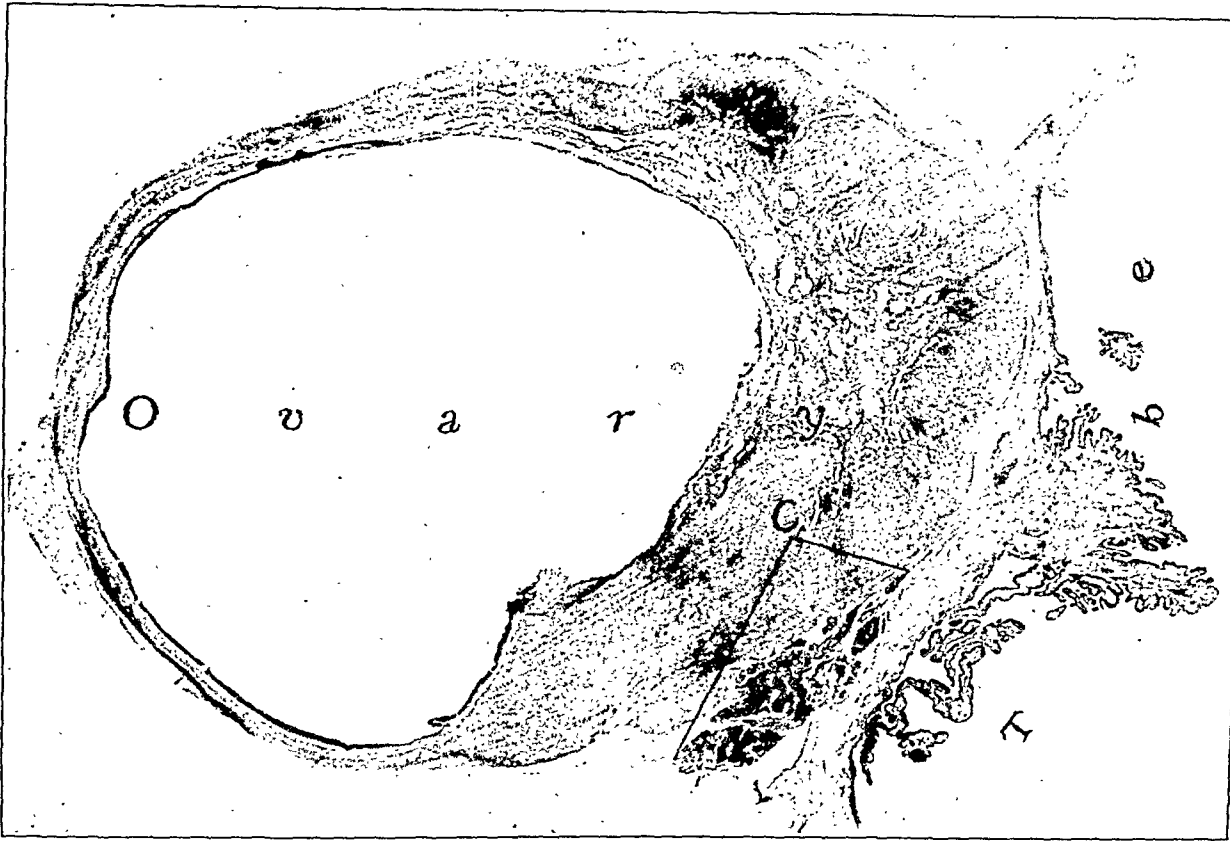


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PLATE 26

FIG. 73. Photomicrograph of a section of the right ovary and portion of the distal end of the occluded tube fused with the surface of the ovary, as may occur in hydrosalpinx (Case 11). Cancer "c" is present in the newly formed tissue which has developed between the ovary and wall of the tube. The small cyst of the ovary is lined by carcinoma. Compare with Fig. 108. $\times 5$.

FIG. 74. Photomicrograph of a section of a portion of the ovary and the occluded fimbriated end of the tube which is fused with the ovary. Carcinoma developing in the fimbriae of the tube might have caused an infolding of these structures and occlusion of the tube, as occurs in inflammatory conditions and also in other instances of carcinoma in this situation (see Fig. 40). The subsequent growth of the carcinoma in this situation might have invaded the ovary and given rise to the conditions indicated in this and the preceding illustration. What was the pathogenesis of the carcinoma of the tubal fimbriae? I believe that it could have arisen from the transtubal migration of particles of cancer escaping from the primary uterine growth. Others may claim that it reached the fimbriae through the lymph or blood streams. See next illustration. $\times 10$.



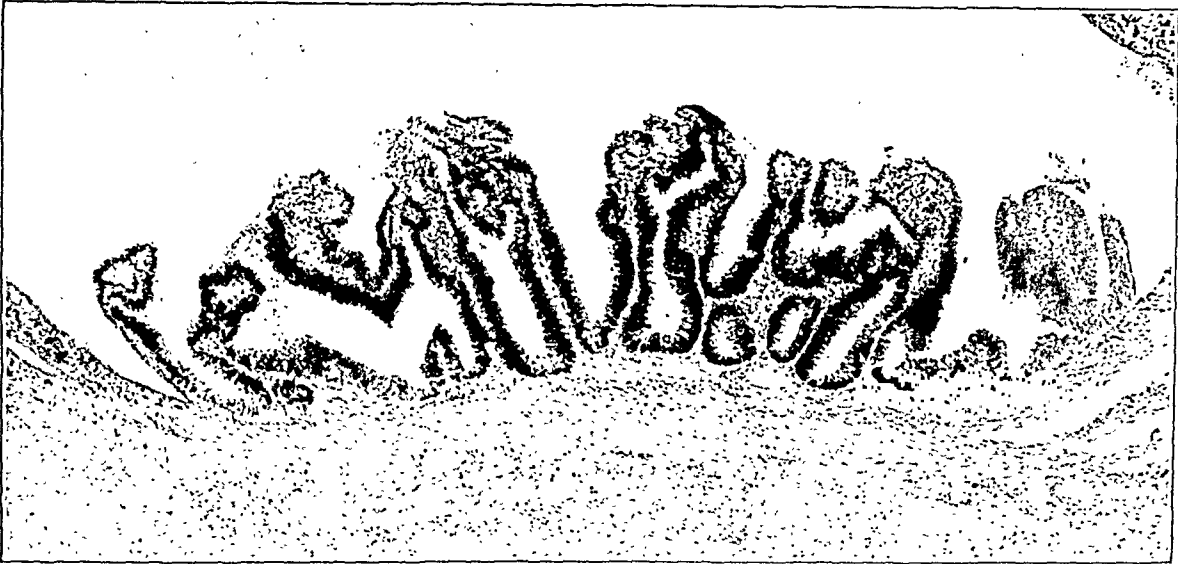
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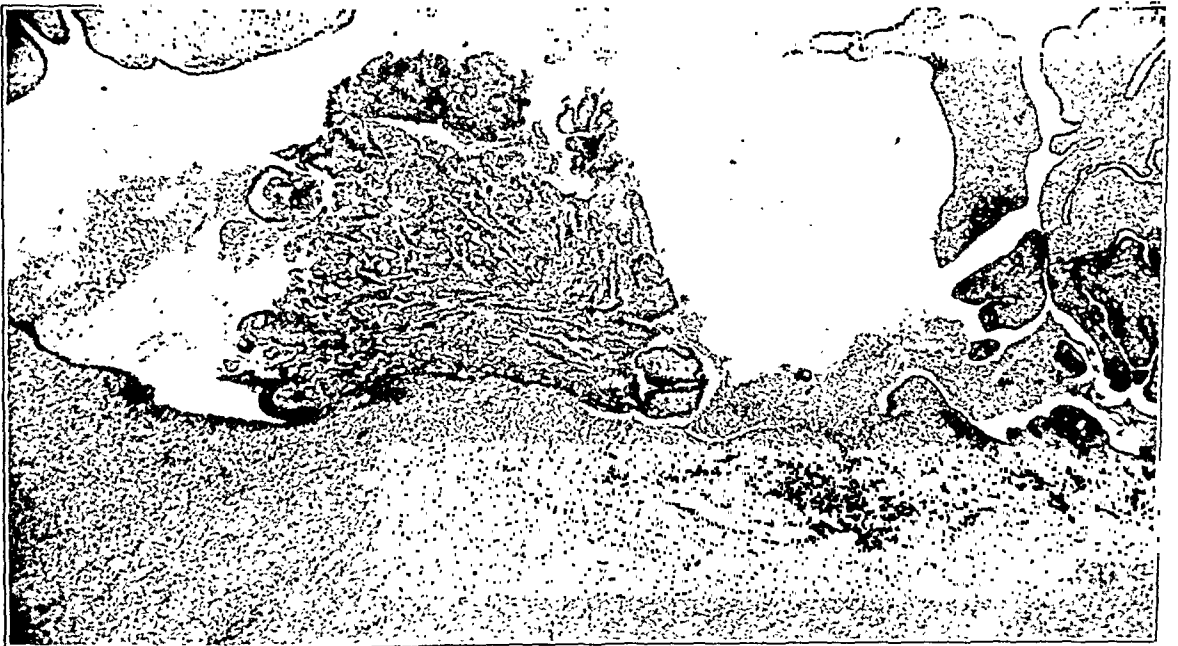
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PLATE 27

- FIG. 75. Photomicrograph of a relatively early patch of carcinoma of the mucosa of the distal portion of the ampulla of the tube. Note the relation of the advancing cancer cells to the normal tubal epithelium on both sides of this patch. The carcinoma probably developed near the center of the patch. I believe that carcinoma starting either as a graft or from a differentiation of tubal epithelium (multicentric origin) could have given rise to the histological picture shown in the sections of this patch of carcinoma. Metastasis through the lymph or blood stream could not have given rise to the condition shown in this or the next photomicrograph. $\times 54$.
- FIG. 76. Photomicrograph of apparently an older patch of carcinoma which has developed in the tubal mucosa. This patch of carcinoma, as the preceding one, was isolated and not continuous with that elsewhere in the specimen. Note the very superficial character of the growth, as though added to the surface of the tubal mucosa. Compare with Figs. 21, 36, 39, 51 and 65. $\times 25$.
- FIG. 77. Photomicrograph (higher magnification) of the section of the tubal wall shown in the preceding photomicrograph, to the right of the patch of carcinoma. The tubal epithelium is lacking in the center of the photomicrograph. Through this break newly formed tissue has poured into the lumen of the tube. Enmeshed in this newly formed tissue are clumps of epithelial cells. It is impossible to state whether they are cancer cells or clumps of hyperplastic desquamated tubal epithelium. Naturally the question arises as to what could have caused this reaction, other than the carcinoma in the tube or something derived from it. This area might provide ideal soil for the grafting of cancer cells floating about in the lumen of the tube. $\times 130$.



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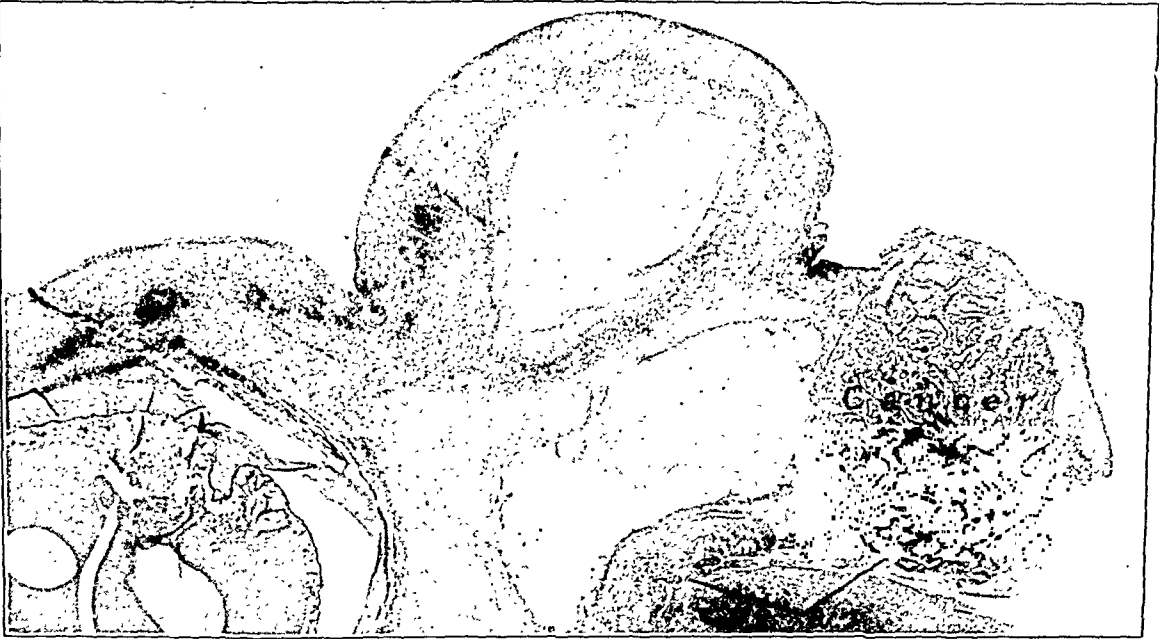
PLATE 28

FIGS. 78 and 79. Photomicrographs of sections of two metastatic carcinomas of the left ovary, associated with an adenocarcinoma of the body of the uterus (Case 12). The bottom of the posterior cul-de-sac was obliterated by a similar growth fusing the posterior wall of the cervix with the sigmoid and presenting in the posterior vaginal vault, as frequently occurs in endometriosis in this situation. Carcinoma in tissue spaces and possibly lymphatics of the ovary was found only about the periphery of the two metastatic tumors, and was judged to represent invasions by the metastatic tumors and not necessarily indicate their pathogenesis. The superficial character of the ovarian metastases, as well as the obliteration of the bottom of the cul-de-sac by the carcinoma, strongly favors the dissemination of carcinoma through the patent tubes with implantation on the surface of the ovary and peritoneum of the cul-de-sac. Exact duplication of the conditions found in this case are frequently encountered in endometriosis. $\times 10$.

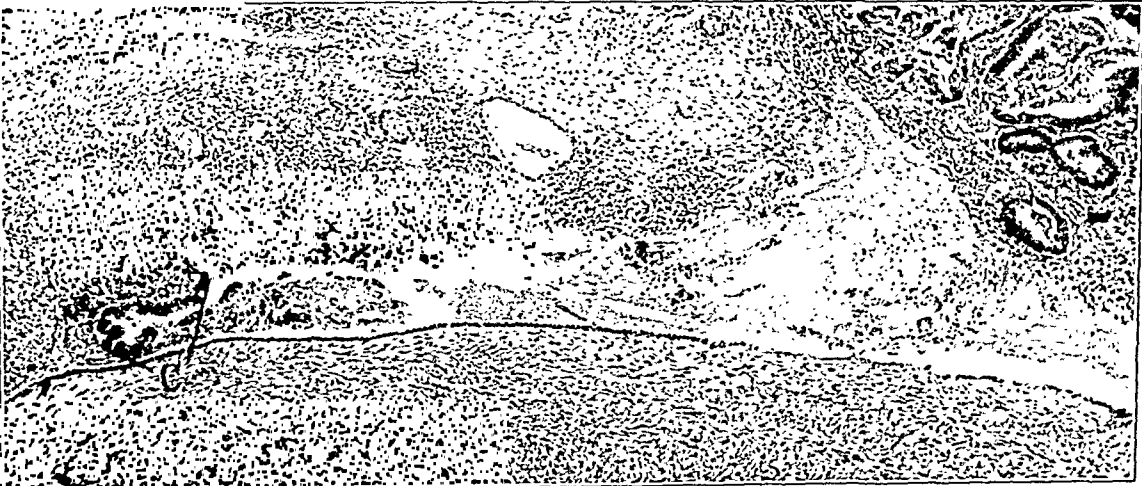
FIG. 80. Photomicrograph (higher magnification) of area "a" indicated in preceding photomicrograph. The character of the metastatic tumor, identical with that present in the uterus, is shown at the right. To the left are cells "c" on the surface of the ovary similar to those of the metastatic tumor, and associated with a definite reaction of the underlying ovarian tissue similar to that caused by an irritant applied to the surface of the ovary. It well might represent the implantation of cancer cells in this situation. $\times 54$.



78



79



80

PLATE 29

FIG. 81. Photomicrograph of a section of a portion of the right uterine cornu including the tube and a small part of the uterine mucosa (Case 13). The patient had a large endometrial tumor distending the uterine cavity and protruding through the cervical canal. This was judged to be a stromal cell sarcoma, or possibly an atypical carcinoma. A papillary adenocarcinoma was also present in other portions of the uterine mucosa. There was a general peritoneal sarcomatosis, including the involvement of the omentum. The normal appearing tube is shown in cross-section with clumps of cells lying free in its lumen which might be malignant. At "c" is a small portion of the uterine mucosa replaced by a papillary adenocarcinoma. At "a" and "b" are two subperitoneal or peritoneal metastases, which appear to be invading the peritoneum from within. Clumps of cancer cells are present in lymphatics beneath the base of the larger tumor. This does not prove the pathogenesis of the metastasis since an implant might have the same potentialities of lymphatic invasion as a primary tumor. While implantation metastases cannot be excluded the whole picture suggests to me metastases through the lymph channels. Compare with "a" of Fig. 1 and "s" of Fig. 7. $\times 10$.

FIG. 82. Photomicrograph (higher magnification) of a portion of the larger metastasis shown in the preceding illustration. Note the malignant tumor in the lymphatic "L," but more important the apparent growth of the tumor towards the peritoneum from within. Compare with Figs. 3 and 11, and also with Figs. 84, 85 and 87. $\times 54$.



81



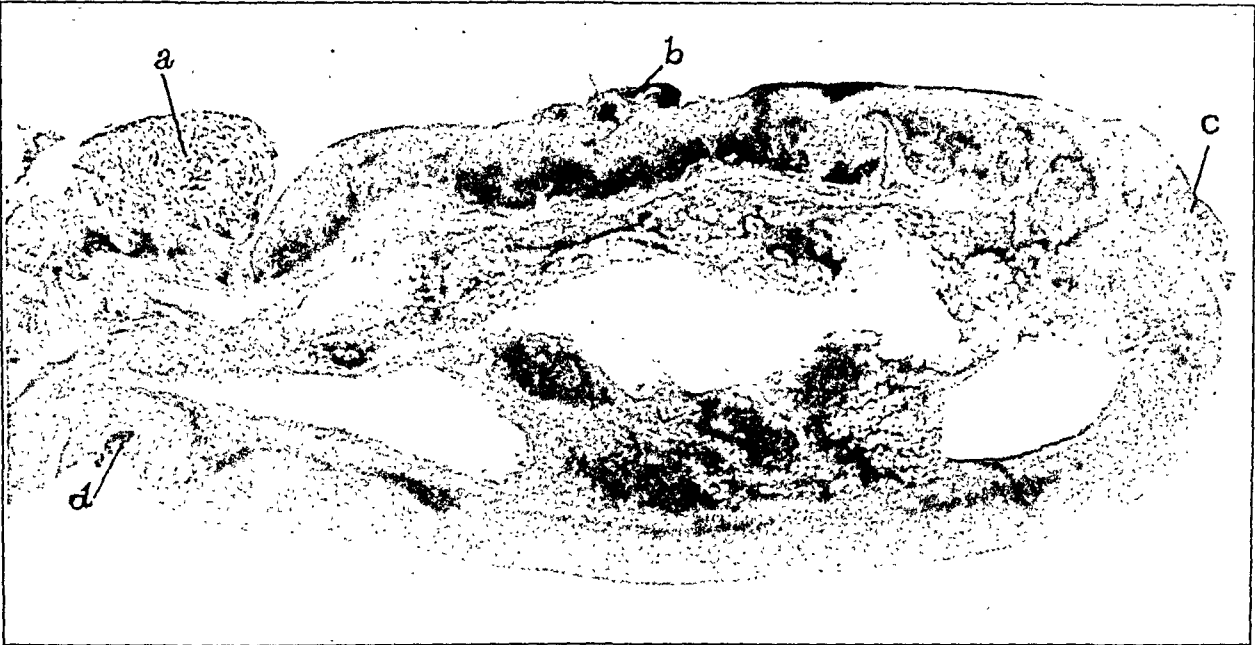
82

PLATE 30

FIG. 83. Photomicrograph of a section of the ovary (Case 13). Metastases "a," "b," "c" and "d" are present on the surface of the ovary without involvement of the lymph vessels of that organ. The ovarian tissue, beneath the base of the largest metastasis "a," has been invaded by the tumor. In all the other three metastases the entire tumor is in newly formed tissue on the surface of the ovary. Similar metastases were present on the surface of the opposite ovary and were all a part of a general peritoneal sarcomatosis, all of which had the same histological structure as that of the uterine tumor. These metastases differ in their histological structure from those of the uterine cornu shown in Fig. 81. These are supraperitoneal and the others are subperitoneal. $\times 8$.

FIG. 84. Photomicrograph (higher magnification) of metastasis "d" of the preceding photomicrograph. Note the very superficial character of the growth, without any evidence of the tumor in the deeper tissues of the ovary. The tumor cells must have been added to the surface of the ovary or have arisen from a differentiation of its surface epithelium, *i.e.*, of multicentric origin. $\times 54$.

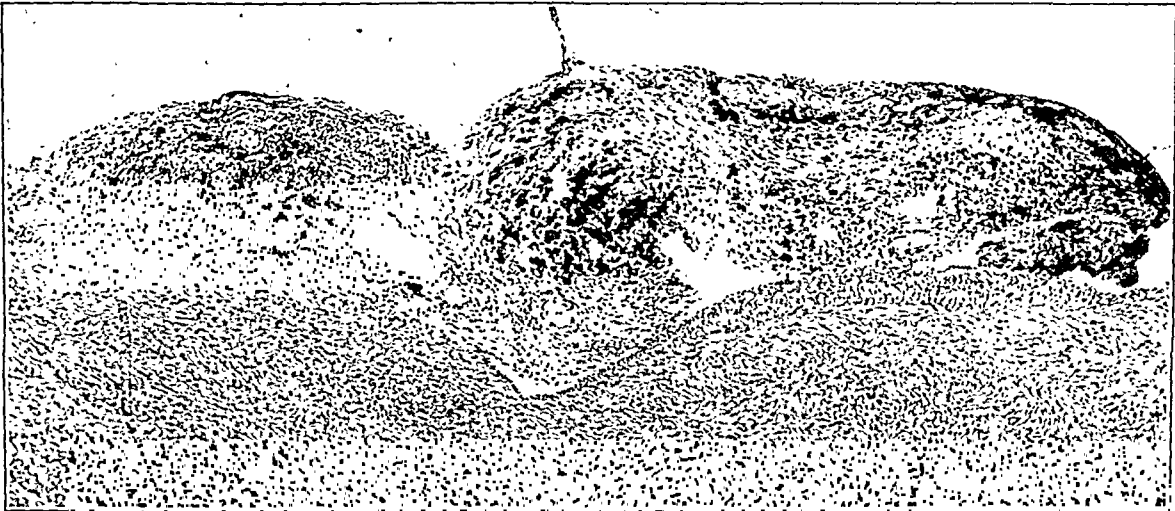
FIG. 85. Photomicrograph (higher magnification) of metastasis "b" indicated in Fig. 83. The entire tumor is situated in newly formed tissue which has developed on the surface of the ovary. The tumor cells are encapsulated in this tissue as foreign bodies become encapsulated on the surface of the peritoneum. Compare with Figs. 16 and 17 where a somewhat similar lesion has resulted from the direct extension of carcinoma through the uterine wall. $\times 25$.



83



84



85

PLATE 31

FIG. 86. Photomicrograph of a cross-section of the ampulla of the tube demonstrating metastases to its peritoneum, similar to those found on the ovaries and elsewhere throughout the peritoneal cavity. Metastasis "a" is very superficial with only a slight invasion of the underlying peritoneum, which is only slightly thickened. In metastases "b" and "c" the peritoneum is markedly infiltrated with carcinoma and therefore greatly thickened, and might be confused with metastases of lymphatic origin. Metastasis "d" has developed in newly formed tissue and is attached by a slender pedicle of the same to the peritoneum of the mesosalpinx. Carcinoma was not found in the lymphatics of the mesosalpinx or of the tubal wall. $\times 8$.

FIG. 87. Photomicrograph (higher magnification) of the metastasis "d." I believe that the pathogenesis of this lesion is the same as that of similar implantation metastases found in peritoneal carcinomatosis of ovarian origin and well shown in Figs. 36 and 37 of a previous paper.⁹ $\times 54$.



86



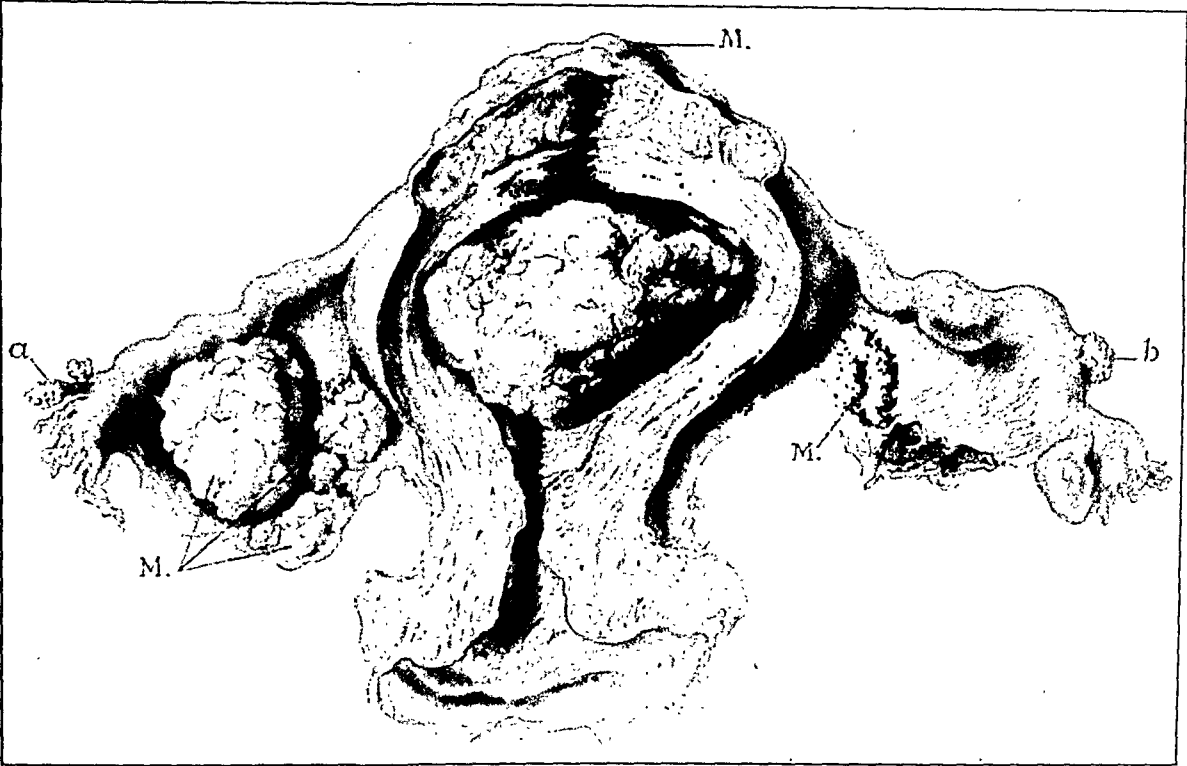
87

PLATE 32

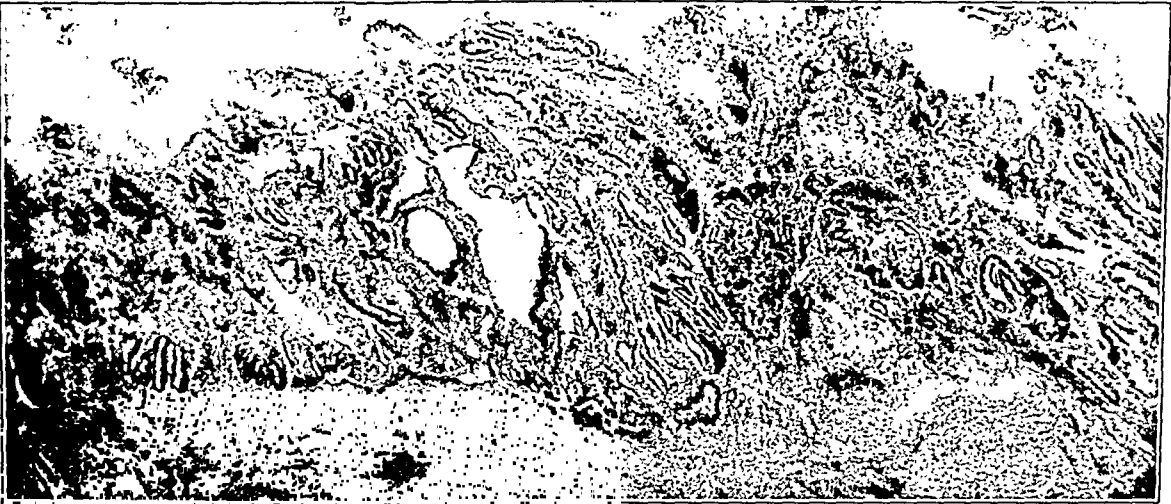
FIG. 88. Uterus and tubes (ovaries are not visible as they lie behind the tubes and broad ligament) of an advanced adenocarcinoma of the body of the uterus, associated with an extensive peritoneal carcinomatosis including the omentum and diaphragm, as in extensive peritoneal carcinomatosis of ovarian origin (Case 14). Peritoneal metastases "M," "M" and "M" are present on the surfaces of the uterus and both broad ligaments. At "a" is a metastasis of the fimbriae of the right tube and "b" a metastasis to the peritoneum of the left tube. The judged primary tumor arose from the mucosa of the posterior wall of the uterus and has distended the uterine cavity and spread into both uterine cornua. $\times 2/3$.

FIG. 89. Photomicrograph of the uterine mucosa at one side of the main tumor, a typical papillary adenocarcinoma. Since this extended into both uterine cornua particles of the carcinoma could have gained access easily to the uterine ostia of the tubes. $\times 54$.

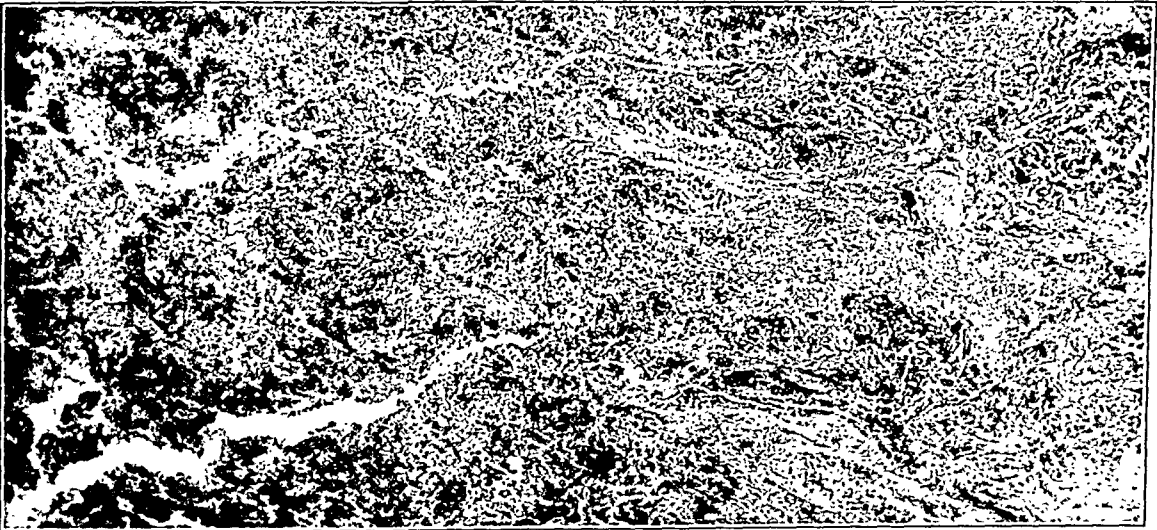
FIG. 90. Photomicrograph of a section of the uterine wall beneath the main tumor. It has been almost entirely replaced by the growth, which is partly necrotic. $\times 54$.



88



89



90

PLATE 33

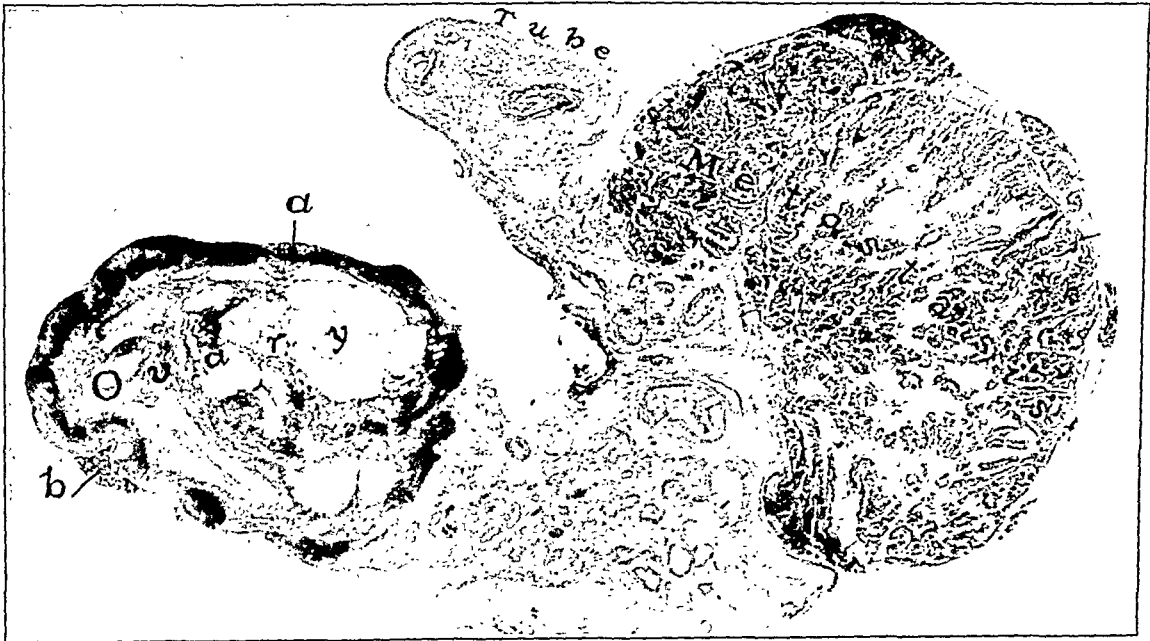
FIG. 91. Photomicrograph of a section of a portion of the uterus beneath the tumor shown in Fig. 88. Since the main tumor was necrotic and very friable the greater portion of it became detached from the uterine wall in cutting the blocks and therefore does not appear in this section. Note the extensive infiltration of the uterine wall by the carcinoma and the encapsulated growth on the peritoneal surface of the uterus to the right. Although many sections from this block were studied I was unable to demonstrate a continuity between the growth in the uterine wall and that on its peritoneal surface. I believe that the latter was of metastatic origin, as is the metastasis shown in the next illustration. Both were a part of an extensive peritoneal carcinomatosis. $\times 3$.

FIG. 92. Photomicrograph of a section through the right ovary, the tube, and the large metastasis of the anterior layer of the right broad ligament (see "M" of Fig. 88). Carcinoma was not found in any of the lymphatics. Therefore, lymphatic permeation can be excluded. In this section carcinoma is present only in the metastasis of the peritoneum of the anterior layer of the broad ligament and on the surface of the ovary at "a" and "b" (see also Figs. 94 and 95). The histological structure of the metastasis on the surface of the broad ligament and that of the peritoneum of the uterus, shown in the preceding illustration, is the same and I believe that both of them had a like origin, beginning in the same way as the metastasis shown in Fig. 101. $\times 3$.

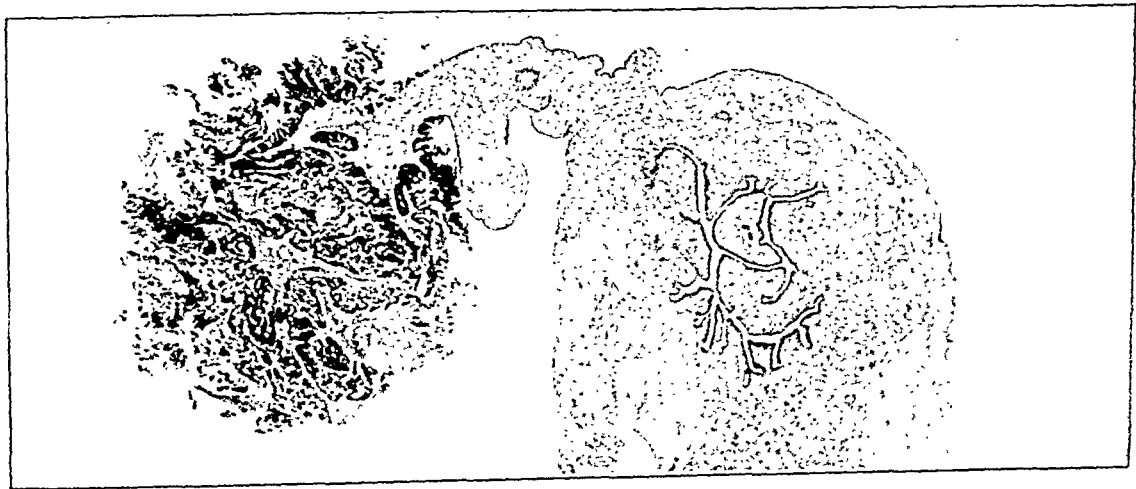
FIG. 93. Photomicrograph of a section of the distal end of the right tube including the carcinoma of the tubal fimbriae shown in "a" of Fig. 88. Did the carcinoma of the fimbriae arise from a differentiation of tubal epithelium or from the implantation of cancer cells on the surface of the fimbriae? I believe the latter, but realize that it cannot be proved. It is a non-encapsulated implant, *i.e.*, of the grafted and not the foreign body type. Compare with Figs. 96, 99 and 100. $\times 10$.



91



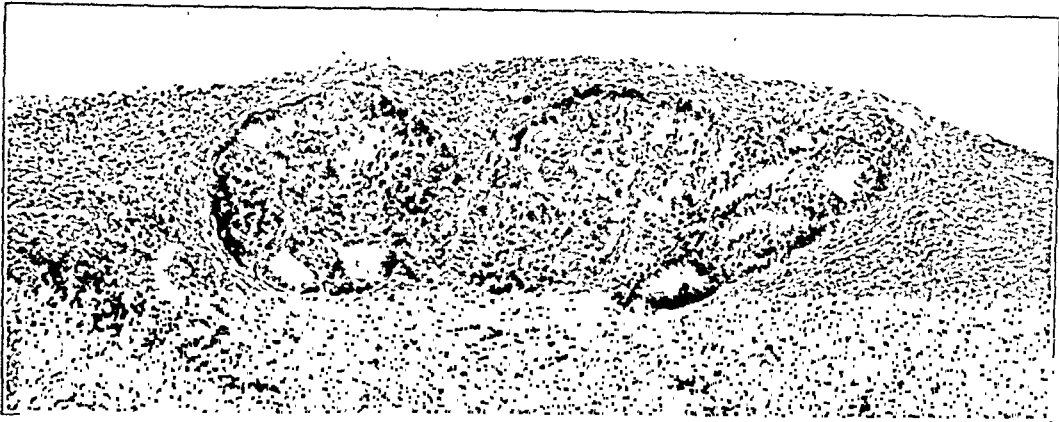
92



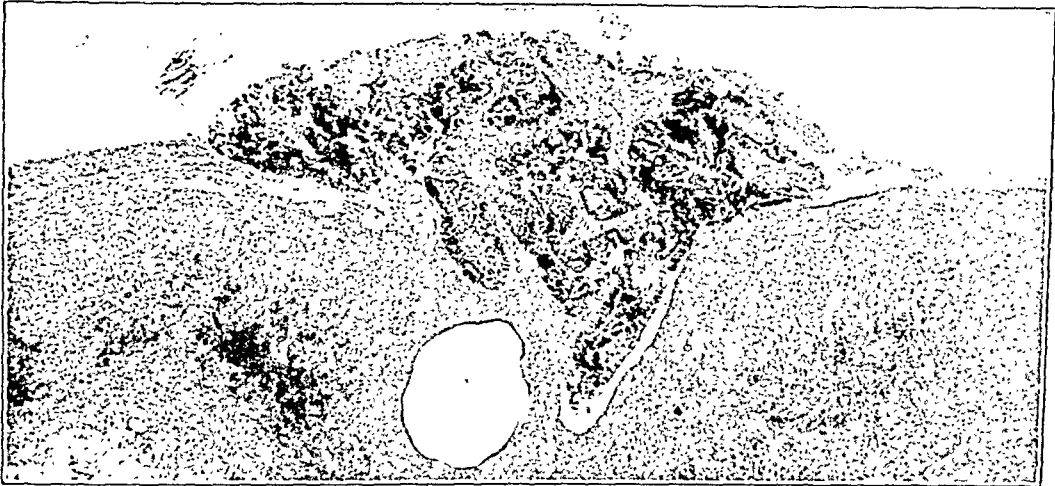
93

PLATE 34

- FIG. 94. Photomicrograph (higher magnification) of the metastasis to the right ovary indicated by "a" of Fig. 92. It is an encapsulated metastasis and might be assumed to be of lymphatic origin. Carcinoma, however, was not found in the lymphatics of the ovary. The encapsulation is identical with that which follows the escape of foreign bodies into the peritoneal cavity, thus suggesting that this metastasis is of implantation origin. See Fig. 2 of previous paper,⁹ also compare with Fig. 96 of present paper. $\times 54$.
- FIG. 95. Photomicrograph (higher magnification) of the metastasis of the right ovary indicated by "b" of Fig. 92. It is a partially encapsulated metastasis and could be also of implantation origin. See next illustration. $\times 25$.
- FIG. 96. Photomicrograph of a section of a portion of the wall of the sigmoid and its mesentery (Case 14). The two metastases have the same histological structure as those of the ovary shown in the two preceding illustrations and, I believe, had a like origin. $\times 25$.
- FIG. 97. Photomicrograph of a section of a portion of the wall and lumen of the right tube showing clumps of cancer cells "C" lying free in the lumen. It is impossible to state whether they entered the tube through its abdominal or uterine ostium. Carcinoma of the mucosa was not present in this tube. Whatever their origin, it demonstrates the transtubal migration of cancer cells. Unfortunately, sections were not made of the uterine ostia of the tubes, but the growth occupied both uterine cornua. $\times 54$.



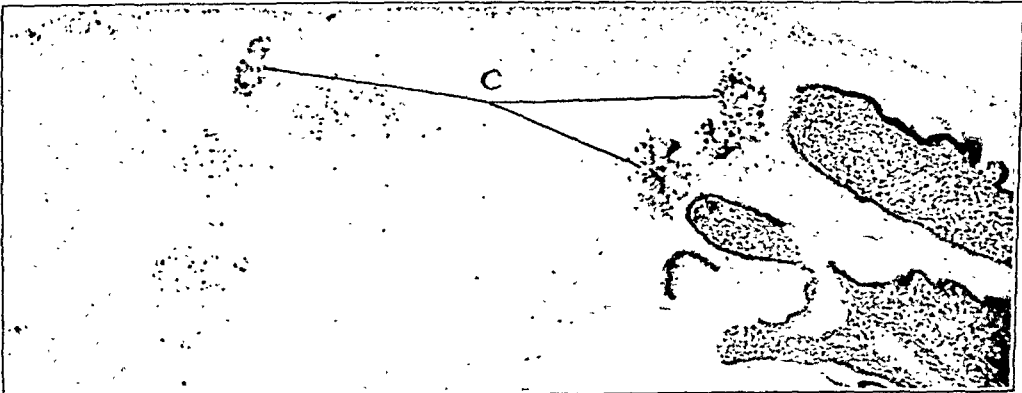
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95



96

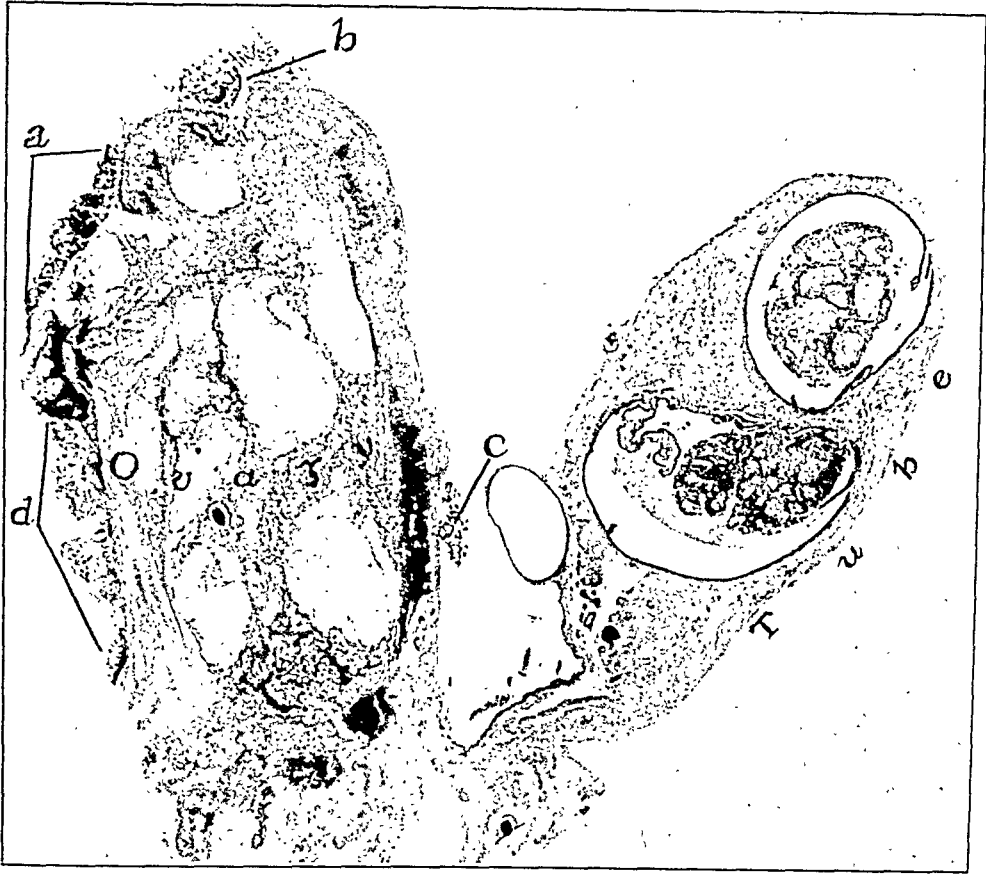


97

PLATE 35

FIG. 98. Photomicrograph of a cross-section of the left ovary and tube shown in Fig. 88. Metastatic carcinoma is present on the surface of the ovary as indicated by "a," "b," "c" and "d." For a higher magnification of implants "a" and "c" see Figs. 103 and 104. These implants are very superficial and without invasion of the deeper ovarian tissue. Two of the three secondary carcinomas of the tube are shown. All are of about the same size and attached to the tubal mucosa by slender pedicles (see Fig. 100). Carcinoma was not found in any of the lymph vessels of the ovary, tube or mesosalpinx. $\times 5$.

FIGS. 99 and 100. Photomicrographs of two cross-sections of the tube from a series of sections taken through metastasis "b" of Fig. 88. The peritoneal metastasis is attached to the surface of the peritoneum by a pedicle, as shown in Fig. 99. The tubal carcinoma is attached to the tubal mucosa by a pedicle "p" of Fig. 100. Carcinoma was not found in the lymphatics of the tube. Although the peritoneal metastasis is encapsulated the growth has penetrated the capsule in various places. The tubal tumor is non-encapsulated and is approximately of the same size as that on the peritoneum. It would seem that they probably had a simultaneous and like origin. The peritoneal tumor has the histological structure of an implantation tumor of foreign body type and that of the tube an implantation tumor of grafted type (see Figs. 101 and 102). The only other rational explanation for the pathogenesis of the two tumors is that they are of multicentric origin. $\times 10$.



98



99

Sampson



100

Carcinoma of Tubes and Ovaries

PLATE 36

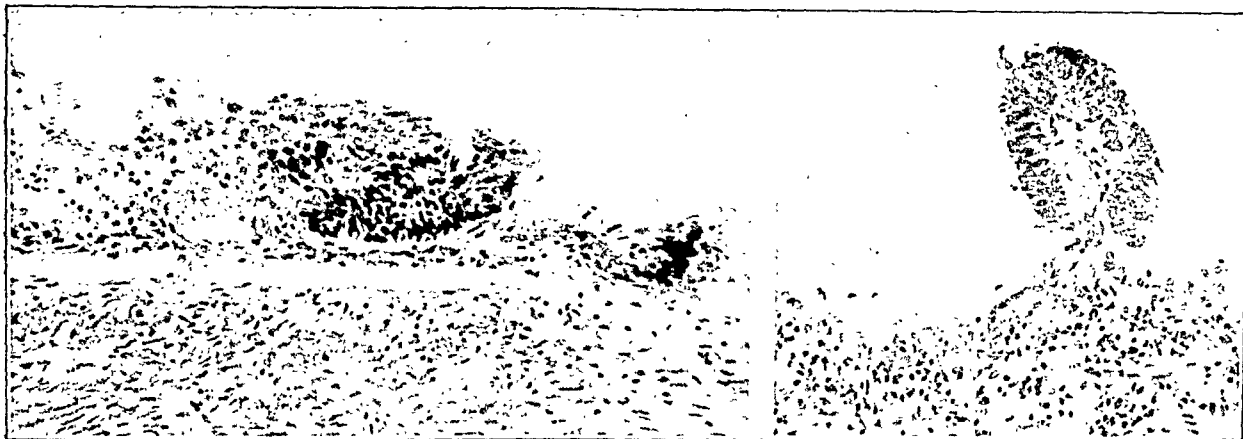
FIG. 101. Photomicrograph of an early implantation of cancer cells on the surface of the ovary between "b" and "c" of Fig. 98. Serial sections made of this portion of the block demonstrated that the carcinoma shown here was not continuous with that elsewhere. It represents a section of the largest part of the growth. A typical reaction to a foreign body is shown; the cancer cells in the center are enmeshed in newly formed tissue, which has arched over the surface of the ovary, while those to the right are becoming encapsulated on the surface of the ovary. Cancer cells might have escaped from any of the nearby exposed peritoneal implantations and lodged on the surface of the ovary in this situation. A later stage of the condition shown here might resemble the growth shown in Fig. 103, and also in Figs. 91 and 92. $\times 130$.

FIG. 102. Photomicrograph of an early implantation on the upper surface of the mesosalpinx shown in Fig. 98 from another section of the same block. This too is not continuous with carcinoma elsewhere. It is non-encapsulated and is either an implantation of grafted type or the result of a differentiation of the mesothelium in this situation. The former theory appeals to me as being the more likely. A later stage of this implant might resemble those shown in Figs. 93, 100 and 104. Compare also with Figs. 21, 36, 39, 51 and 65. $\times 130$.

FIG. 103. Photomicrograph (higher magnification) of carcinoma on the surface of the left ovary, indicated by "a" of Fig. 98. It might have begun as shown in Fig. 101 and subsequently spread over the surface of the ovary. If not sufficiently checked by encapsulation implantations similar to those shown in Figs. 96, 101 and 102 might arise from it. One can see that clumps of cancer cells would easily escape into the peritoneal cavity from the surface of such an implant and might give rise to secondary implantations of the growth. $\times 54$.

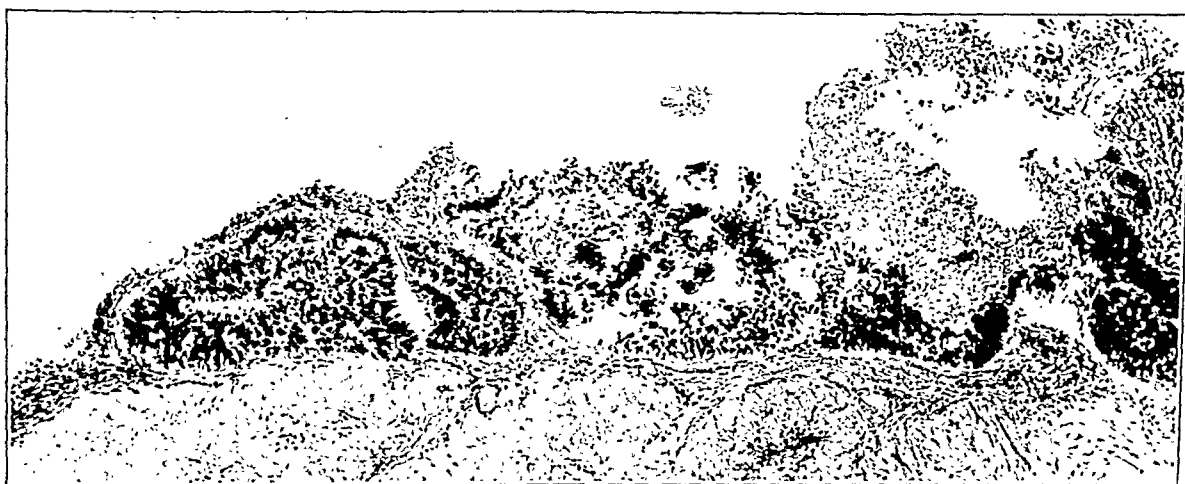
FIG. 104. Photomicrograph (higher magnification) of implant "c" of Fig. 98, but from another section of the block. One can realize that this might have begun as the implant shown in Fig. 102. Compare also with Fig. 95. $\times 25$.

FIG. 105. Photomicrograph of a portion of the surface of one of the tubal carcinomas shown in Fig. 96, and also of the tubal mucosa opposite it. Is the carcinoma of the mucosa a contact implant or one of multicentric origin? I was unable to demonstrate that it was continuous with the growth above it. $\times 54$.



101

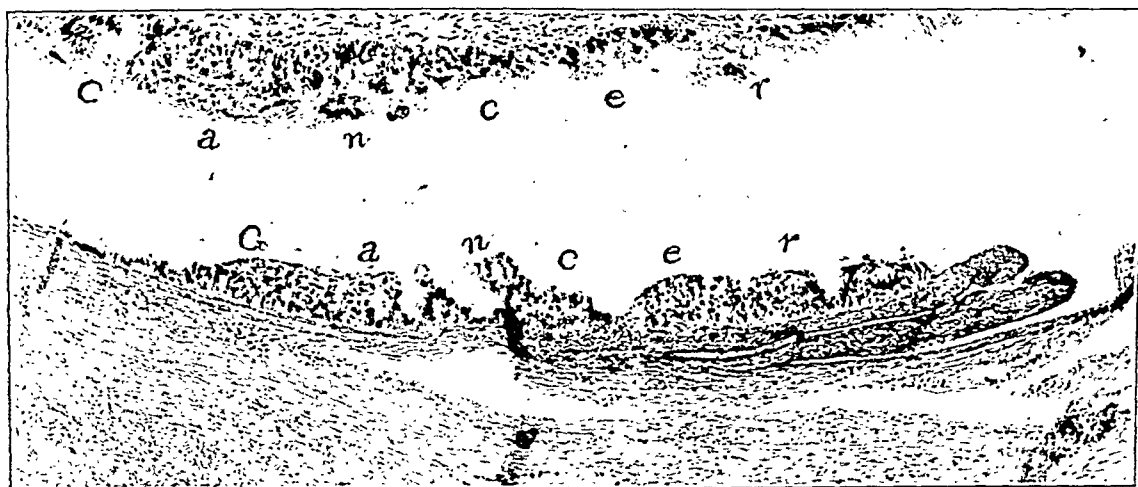
102



103



104



105

PLATE 37

FIG. 106. Photomicrograph of a section of a portion of the uterine mucosa from a patient (Case 15) with adenocarcinoma of the body of the uterus treated with radium 2 months before. Carcinoma is still present in the deeper portion of the endometrium. This was found only in a few places. Carcinoma was not found in the uterine wall (many sections from several blocks were examined). $\times 54$.

FIG. 107. Photomicrograph of a section of a portion of the right ovary showing a judged metastatic adenocarcinoma of that organ secondary to that of the uterus. What is the pathogenesis of this carcinoma of the ovary? This photomicrograph is of a portion of the wall of the small ovarian cyst shown in the next illustration, but from another section of the series. $\times 54$.

FIG. 108. Photomicrograph of a cross-section of the right tube and ovary. The tube appears normal. It was patent, but presented no particles of carcinoma in its lumen. Carcinoma, apparently in patches "c," "c," "c," "c," is scattered on the surface of the ovary. A small malignant ovarian cyst is present, the epithelial lining of which is continuous with that of the growth on the surface of the ovary. Carcinoma is enmeshed in newly formed tissue which has arisen on the surface of the ovary between it and the tube. Serial sections demonstrated that the majority, if not all, of the patches of carcinoma on the surface of the ovary composed one tumor which had spread over the surface of that organ in an irregular manner. Carcinoma was not found, either in the lymph vessels of the mesosalpinx, or hilum of the ovary. The distribution and general histological picture of the ovarian carcinoma in this case are exact duplications of those frequently encountered in ovarian endometriosis. $\times 5$.

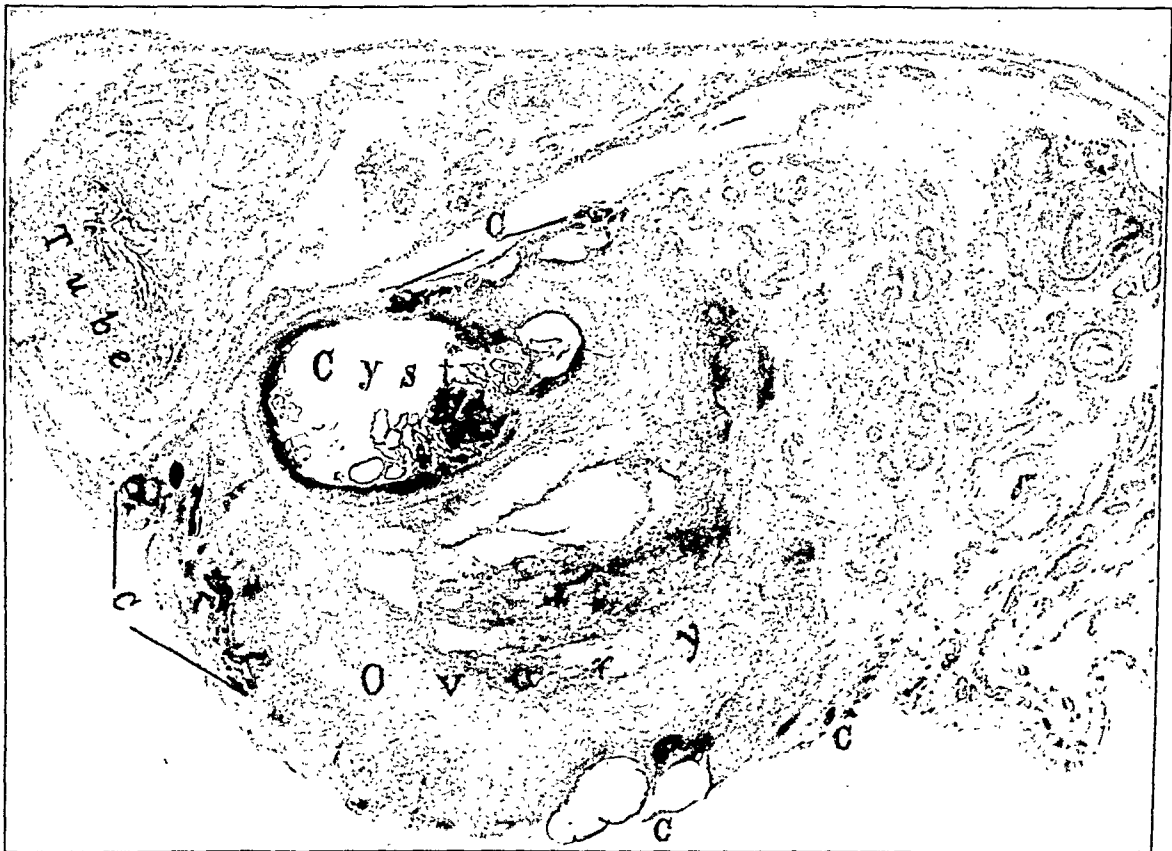
FIG. 109. Photomicrograph of carcinoma enmeshed in newly formed tissue on the surface of the ovary. What is its pathogenesis? Serial sections demonstrated that it was but a part of the advancing edge of the growth in the newly formed tissue on the surface of the ovary between it and the adherent tube of the preceding illustration. $\times 54$.



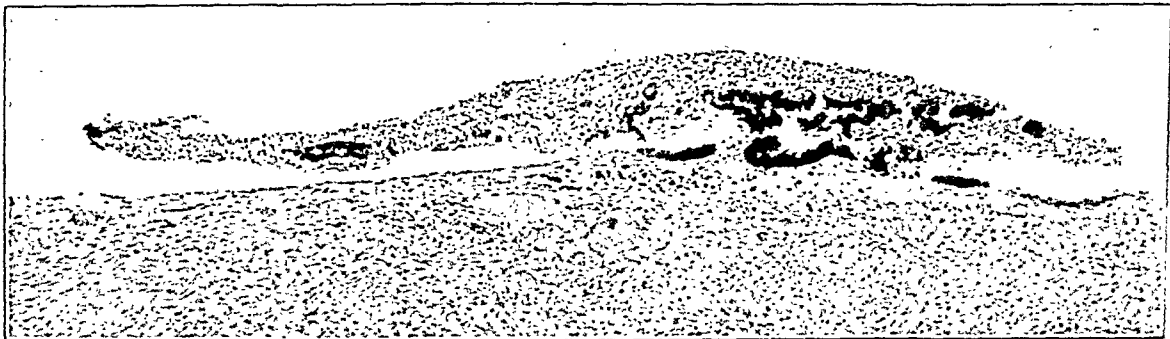
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107



108



109

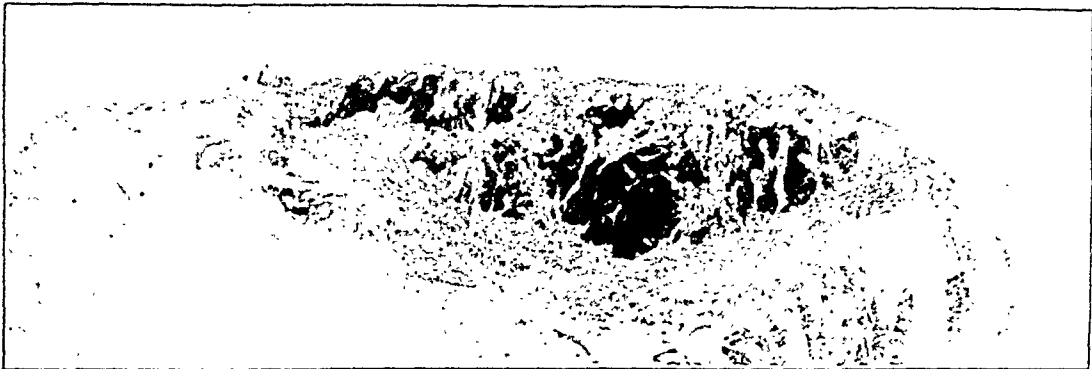
PLATE 38

FIG. 110. Photomicrograph of a section of a peritoneal metastasis in the posterior cul-de-sac. Its general histological structure is similar to that of encapsulated foreign bodies in the peritoneum and likewise that of many of the peritoneal metastases in ovarian carcinoma and of peritoneal endometriosis. The growth in this situation had invaded some of the subperitoneal lymphatics as a primary carcinoma invades the lymphatics of the organ in which it arises. $\times 10$.

FIG. 111. Photomicrograph of a section of a portion of the anterior wall of the uterus and (to the right) the uterovesical reflection of peritoneum. The surfaces of both are covered with carcinoma. At operation the uterovesical reflection of peritoneum was found to be fused with the lower portion of the anterior wall of the body of the uterus. During the operation the adherent peritoneum was pulled away from the anterior wall of the uterus, thus exposing the carcinoma which had developed in the bottom of the anterior cul-de-sac and fused its peritoneum to the anterior wall of the uterus. Similar conditions are frequently found in this situation in both peritoneal carcinomatosis of ovarian origin and in peritoneal endometriosis. $\times 5$.

FIG. 112. Photomicrograph of a cross-section of the tip of an epiploical appendage of the sigmoid, which was fused with the peritoneum of the posterior cul-de-sac by carcinoma uniting the surfaces of the two structures. They were separated at the operation. The appendage and the corresponding area of the peritoneum were excised. What is the pathogenesis of these multiple patches of carcinoma on the surface of the ovary and peritoneum? The capsules containing radium, which were introduced through the cervix into the uterine cavity like the plunger of a piston syringe, easily could have forced blood-containing particles of cancer out through the patent tubes into the peritoneal cavity. The subsequent implantation of these particles of cancer would account for the metastases found at operation 2 months after the application of radium. $\times 10$.

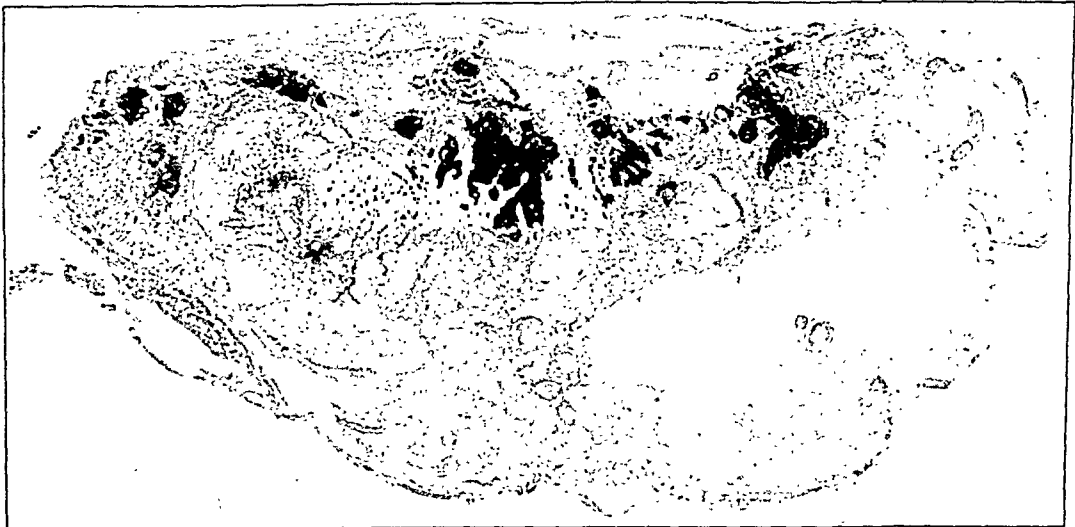
FIG. 113. Photomicrograph of a section of the metastasis found in the scar of the abdominal incision 4 months after the uterus, tubes and ovaries were removed, and the peritoneal implants shown in the preceding illustration were excised. The metastasis is situated just beneath the skin, where the edges of the abdominal wall were approximated in the closure of the abdominal incision. Particles of cancer might easily have been implanted in the abdominal wound during the excision and removal of the peritoneal metastases. There was no induration of the abdominal wall beneath the scar. The strongest possible circumstantial evidence indicates that this metastasis arose from the successful implantation of cancer in the wound of the abdominal incision during the operation, just as the other judged implantation metastases shown in this paper arose from the lodging and retention of cancer cells in a wound, with the attempted healing of the wound and the continued growth of the carcinoma in its new situation. $\times 6$.



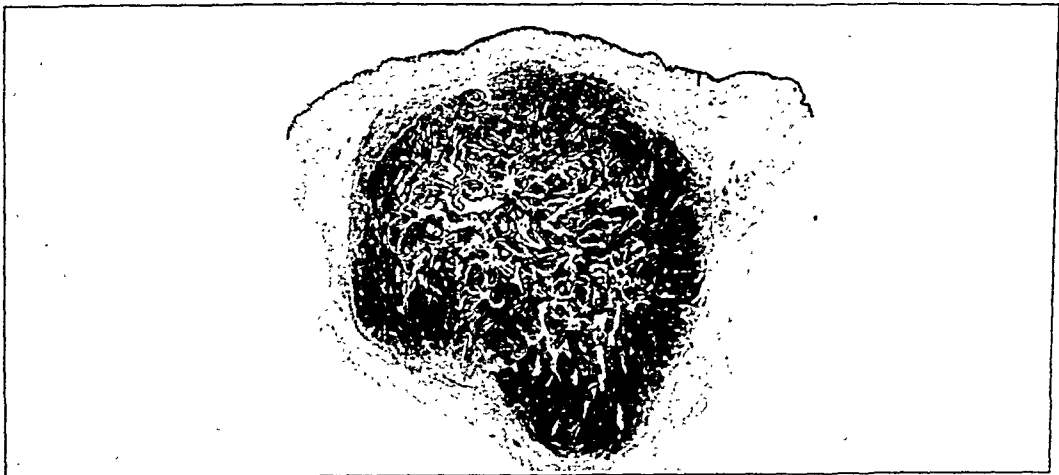
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111



112



113

STUDIES ON THE MATURE AND IMMATURE LYMPHOID CELLS OF SPLEEN, LYMPH NODES AND THYMUS OF NORMAL RATS AND RATS INFECTED WITH TRYPANOSOMA BRUCEI *

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In a previous histological study on the lymphatic reaction in experimental trypanosomiasis † it was found that in the spleen and lymph nodes of white rats infected with *Trypanosoma brucei* there was, besides increased activity in the hematopoietic and reticulo-endothelial systems, a very striking lymphoblastic hyperplasia. Many of the large lymphoblasts that appeared in the early stage of infection were found to be formed by hypertrophy of the small round cells (ordinarily known as small lymphocytes), which constitute the main bulk of these organs. Not all of the small round cells, however, underwent hypertrophy, many of them remaining small even at the end of infection. Furthermore the small round cells of the thymic

* Received for publication May 12, 1933.

† Since the original report may not be easy of access to all readers of this article, an abstract is given here.

In the sections of normal rat spleen, lymph nodes and thymus, stained with Mallory's eosin-methylene blue stain, five varieties of cells can be differentiated as follows: (1) Large and medium sized lymphoblasts with intensely basophilic cytoplasm and clear nuclei with large irregular chromatin masses. These cells are found not only in the germinal centers, but also scattered among the small lymphoid cells. (2) Large and medium sized lymphocytes with only faintly basophilic cytoplasm. These are also widely scattered, but are especially numerous in the outer zones of the lymph follicles of the spleen. (3) Small lymphoid cells with very scanty cytoplasm and small round nuclei. These form the main bulk of the lymphoid organs. (4) Plasma cells with violet cytoplasm and eccentric nuclei. These are absent in the splenic lymph follicles of normal rats but are very numerous in the medulla of the lymph nodes — especially those of the chest and abdominal cavities. (5) Cells of the reticulo-endothelial system, which have acidophilic cytoplasm, fine nuclear chromatin masses and thin nuclear membrane, and can therefore be distinguished easily from all the above varieties.

In the experiment the rats, after they were each inoculated intraperitoneally with about 10,000 trypanosomes, died in from 7 to 10 days. By killing them on successive days after inoculation it was possible to follow the series of changes taking place in the lymphoid organs. Forty-eight hours after inoculation the spleen was found to be already greatly enlarged, and in sections many large lymphoblasts had appeared in the interfollicular lymphoid tissue where normally only a few such cells were present. At this stage it was also found that there were many intermediate forms between the large lymphoblasts and the small lymphoid cells. Especially significant was the scarcity of mitotic figures and the slightly increased amount of dark blue cytoplasm around the

cortex, which are also considered by many to be small lymphocytes, did not hypertrophy. We therefore assumed that these small round cells, which we designated as small lymphoid cells, could be divided into two types, according to their ability to hypertrophy: one, the small primitive cells which can form large lymphoblasts; the other, the true small lymphocytes which cannot form large lymphoblasts. Since these two types cannot be differentiated from each other in sections stained by the usual methods, the present study was made in an attempt to differentiate them by using the supravital technique.

MATERIAL AND METHODS

White male rats from 5 to 6 months old, raised in China, were used. Occasionally, younger animals were employed. The strain of *Trypanosoma brucei* was kindly supplied by Professor F. G. Novy. A preliminary study showed that the histological pictures of the normal and the infected rats were identical with those previously described.¹

The infective material consisted of citrated blood of an infected rat, diluted 10 to 20 times with normal saline. Each animal to be infected was injected subcutaneously or into the thymus with an amount of the material containing approximately 10,000 or 3000 parasites respectively. In intrathymic injections a small amount of India ink was added to the inoculum as an indicator, and in order to facilitate these injections smaller animals 2 to 3 months old were used.

The cells studied were those of the inguinal lymph nodes, the mesenteric lymph nodes, the spleen and the thymus. Since the inguinal lymph nodes are easy of access and the thymus cannot be exposed without endangering the life of the animal, the inguinal lymph nodes were examined first, then the abdominal lymphoid organs and finally the thymus. All operations were carried out under ether anesthesia. A small bit of the desired tissue was removed with a pair of fine scissors and immediately placed in a drop of rat serum on a clean coverslip, where the cut surface of the tissue was gently scraped with a cataract knife until the serum became slightly cloudy. A small drop of the serum was then trans-

nuclei of some of the small lymphoid cells. The increase of the lymphoblasts at this stage then, was apparently due to the hypertrophy of some of the small lymphoid cells. There were, however, many small lymphoid cells in which no change could be detected.

During the next 2 or 3 days there was a progressive increase in number of both the large, and especially the medium sized lymphoblasts, while the unchanged small lymphoid cells gradually decreased in number. Mitotic figures among the lymphoblasts became very numerous. The plasma cells and the transitional forms between the lymphoblasts and plasma cells also began to appear in the lymph follicles in large numbers. After the 6th day the spleen became less cellular, owing to the reduction in number of the lymphoblasts and plasma cells.

The above described changes were most striking in the spleen and in the abdominal and thoracic lymph nodes. The inguinal lymph nodes showed only moderate lymphoblastic hyperplasia. The thymic cortex showed surprisingly little change, in spite of the fact that marked lymphoblastic hyperplasia was present in the lymph nodes in its immediate neighborhood.

ferred to another coverslip, which was instantly placed on a slide previously coated with neutral red and Janus green. From this film 500 cells were immediately counted. Just before opening the chest cavity to expose the thymus the animal was exsanguinated from the carotid artery. For the cell suspension of infected rats the serums of both normal and infected animals were used, with no apparent difference in results.

DESCRIPTION OF THE LYMPHOID CELLS

It may be said at the outset that the lymphatic change observed in experimental trypanosomiasis of the rat is largely of a quantitative nature, involving the different types of lymphoid cells found under normal circumstances. The following tabular description of the cells (see Table I) is given as a basis for the presentation of the observations under discussion.

OBSERVATIONS ON NORMAL RATS

As a control to the findings obtained on the cells of the lymphoid organs of rats infected with *Trypanosoma brucei* parallel observations were made on normal rats of the same age and sex, fed on the same diet. Before referring to the differential counts in this control series the following points should be mentioned as possible sources of error in work of this type.

1. The mitochondria, which are usually well stained within a few minutes after the film is made, sometimes lose their stain very rapidly without the occurrence of any other noticeable morphological change. The loss of stain is found to be due to overcrowding, for in the films with fewer cells this phenomenon is not observed.

2. Most of the degenerated cells (Fig. 19) are originally small immature lymphocytes. The degenerative change takes place so rapidly that unless the films are examined quickly it will not be observed. In the process of degeneration a clear zone first appears around the nucleus; then the rest of the cytoplasm swells so that it becomes practically invisible. While this swelling of the cytoplasm is taking place the mitochondria, which are sometimes already stained, also swell and begin to dance about, and finally become stainless granules, or what look like vacuoles either diffusely scattered or grouped together on one side of the nucleus, which, in the meantime, has become round and homogeneous but unaltered in its size. The exact cause of the degeneration is not known and the number of degenerated cells does not seem to be appreciably in-

TABLE I

Description of Different Types of Lymphoid Cells Found in the Rat

Cell name	Size ³	Cytoplasm	Mitochondria	Neutral red bodies ⁶	Nucleus
Lymphoblasts	L, M-S, S	Opaque	Coarse and abundant	None or very few	Rounded or irregular, large chromatin masses
Immature lymphocytes	L, M-S, S	Slightly opaque	Medium sized, less abundant	Few	Rounded or irregular
Lymphocytes	L, M-S, S	Clear	Fine, scanty	Few or numerous	
Degenerated cells	L, M-S, S ⁶	Swollen, practically invisible	Usually not seen, but there are unstained vacuoles		Round, homogeneous, nuclear membrane distinct
Plasma cells ¹	L, M-S, S	Opaque	Coarse, medium sized or fine	Few and fine	Usually rounded, eccentric position, large chromatin masses
Monocyte-like cells ²	L, M-S, S	From opaque to clear	From coarse and abundant to fine and scanty	Few or abundant, rosettes	Kidney-shaped, eccentric position

1. There are in the infected animals cells representing the transitional forms between lymphoblasts and plasma cells. Such forms are classified either as lymphoblasts or plasma cells, depending upon the type to which they show the greatest resemblance.

2. It is notoriously difficult in the rat to differentiate monocytes from lymphoid cells. The term "monocyte-like" employed here is used merely to indicate a group of cells having certain morphological characteristics without definitely implying that they belong to another cell strain.

3. The size of the cell is classified as large (L) if the cell diameter is over 12 microns, medium sized (M-S) if it is between 9 and 12 microns, and small (S) if it is between 7 and 9 microns.

4. Some large lymphoblasts may measure 20 or more microns in diameter.

5. Owing to the marked swelling of its cytoplasm the size of the degenerated cell is determined not by its diameter but by the size of its nucleus, using the nuclei of the undegenerated lymphoid cells for comparison. According to this method of determination it was found that most of the degenerated cells were of the small variety.

6. There is a great variation in the amount and arrangement of the neutral red bodies in different cells, which is especially noticeable in the lymphocytes. A classification of the cells according to the arrangement of their neutral red bodies will be given in the following paper²; but as they are found only in small numbers in this study no further differentiation will be made here.

fluenced, either by mechanical pressure, by the action of the stains, or by the age of the preparation.

3. Although many of the small round cells of the thymus are indistinguishable from those of the lymph nodes and spleen there is,

TABLE II

*Average Percentage Values of the Differential Counts on the Organs of 7 Normal Rats:
500 Cells Counted in Each Preparation*

Type of Cell	Organ			
	Inguinal lymph node	Mesenteric lymph node	Spleen	Thymus
Small lymphoblasts	0.6	1.3	2.0	1.1
Small immature lymphocytes	75.7	64.7	60.0	83.9 ²
Small lymphocytes	0.9	1.9	1.9	+
Small degenerated cells	21.2	29.3	28.9	9.7
Medium sized lymphoblasts	0.3	0.4	1.3	2.0
Medium sized immature lymphocytes, lymphocytes and degenerated cells ¹	0.3	1.0	2.7	0.9
Large lymphoblasts	0.2	0.4	0.8	1.1
Large immature lymphocytes, lymphocytes and degenerated cells ¹	0.2	0.3	0.9	0.5
Plasma cells	0	0.4	0.3	+
Monocyte-like cells	+	0	0	0
Clasmatocytes	0.1	0.1	0.6	0.4
Neutrophilic leukocytes	0.1	0.1	0.4	0.1
Eosinophilic leukocytes	+	+	0.3	+

1. Owing to their scarcity these cells are grouped together under their respective sizes.

2. Includes some cells morphologically slightly different (see text).

+ = present, but less than 0.1 per cent.

among the small immature lymphocytes, one type that is much more frequently seen in this organ than in the other organs. Cells of this type (Figs. 10, 11, 12) are characterized by rather deep and coarse neutral red bodies, usually two or three in number, but sometimes more, generally clustered on one side of the cell. The mitochondrial

content is not noticeably different from that of the ordinary small immature lymphocytes.

Differential counts of the cell suspensions from the surface scraping of the inguinal lymph nodes, mesenteric lymph nodes, spleen and thymus of 7 normal animals were made, the results of which are shown in Table II. It will be seen that lymphoblasts of all sizes (Figs. 1, 20, 27) were found in all these organs, although only in small numbers. The majority were the small immature lymphocytes (Figs. 3, 4, 5 and 6) which, together with their degenerated forms, constituted about 90 per cent of the total count. Attention is to be given to the fact that whereas the percentage of small degenerated cells was fairly high in the lymph node and the spleen films, it was much lower in those of the thymus. Mention should also be made of the fact that although myeloid cells were occasionally seen in the films made from splenic tissue, they were not encountered when these particular counts were made.

OBSERVATIONS ON INFECTED RATS

It has been found¹ that intraperitoneal injection of *Trypanosoma brucei* is followed by a rapid lymphoblastic hyperplasia of the spleen and of the lymph nodes of the thoracic and abdominal cavities. The reaction in the inguinal and other superficial lymph nodes, however, is only slight or moderate. The absence of a marked reaction in these lymph nodes is apparently due to the scarcity of parasites in them. To prove this point 7 rats were infected subcutaneously in the right inguinal region. On the 7th day after inoculation it was found that the spleen was as much enlarged as in those animals infected by the intraperitoneal route, but also that there was an extreme enlargement of the inguinal lymph nodes on the side of injection (Fig. 32). They formed a mass, fifteen or twenty times larger than normal, and areas of necrosis and hemorrhage were frequent. In marked contrast to this finding the inguinal lymph nodes on the left side, which was not inoculated, and the thoracic and mesenteric lymph nodes showed only slight enlargement. The results of the cell counts on these animals are shown in Table III.

In Table III it will be noted that the increase in the number of lymphoblasts and plasma cells is most marked in the right inguinal lymph nodes, slightly less in the spleen, much less in the mesenteric

and left inguinal lymph nodes, and almost unnoticeable in the thymus.

The differences between the percentage values of the lymphoblasts and plasma cells of the lymphoid organs of the normal rats

TABLE III

Average Percentage Values of the Differential Counts on the Organs of 7 Infected Rats Examined on the 7th Day of Infection: 500 Cells Counted in Each Preparation

Type of Cell	Organ				
	Right inguinal lymph node	Left inguinal lymph node	Mesenteric lymph node	Spleen	Thymus
Small lymphoblasts	13.3	0.8	1.7	6.0	1.3
Small immature lymphocytes	39.0	66.1	70.0	49.4	82.0 ²
Small degenerated cells	19.1	29.0	22.9	13.8	11.2
Small lymphocytes	0.3	0.1	0.5	0.4	0
Medium sized lymphoblasts	9.8	0.9	1.0	8.3	2.4
Medium sized immature lymphocytes, lymphocytes and degenerated cells ¹	0.9	0.6	0.3	0.9	0.4
Large lymphoblasts	7.0	0.3	0.5	7.9	1.1
Large immature lymphocytes, lymphocytes and degenerated cells ¹ ..	1.0	0.3	0.3	1.9	0.2
Plasma cells	6.5	0.2	1.6	4.2	+
Monocyte-like cells	+	0.2	0	+	+
Clasmatocytes	2.9	1.4	0.8	6.1	1.0
Neutrophilic leukocytes	0.2	0.1	0.1	0.7	0.3
Eosinophilic leukocytes	+	0.3	0.2	0.5	+

1. Owing to their scarcity these cells are grouped together under their respective sizes.

2. Includes some cells morphologically slightly different (see text).

+ = present, but less than 0.1 per cent.

and those of the infected rats, as shown in Tables II and III, are graphically represented in Chart I.

The striking difference in the reaction of the inguinal lymph nodes of two sides indicates that the degree of hyperplasia in the lymphoid tissue is dependent upon the local concentration of parasites. In

order to ascertain if the failure of the thymus to participate in the general reaction is also due to the absence in it of parasites, a mixture of India ink and about 3000 trypanosomes was injected directly into the thymus of 5 rats. By the 5th or 6th day, when the blood was swarming with trypanosomes and the animals were in a moribund state, counts of thymic cells were made. The results of these counts, as shown in Table IV, indicate that lymphoblastic proliferation was

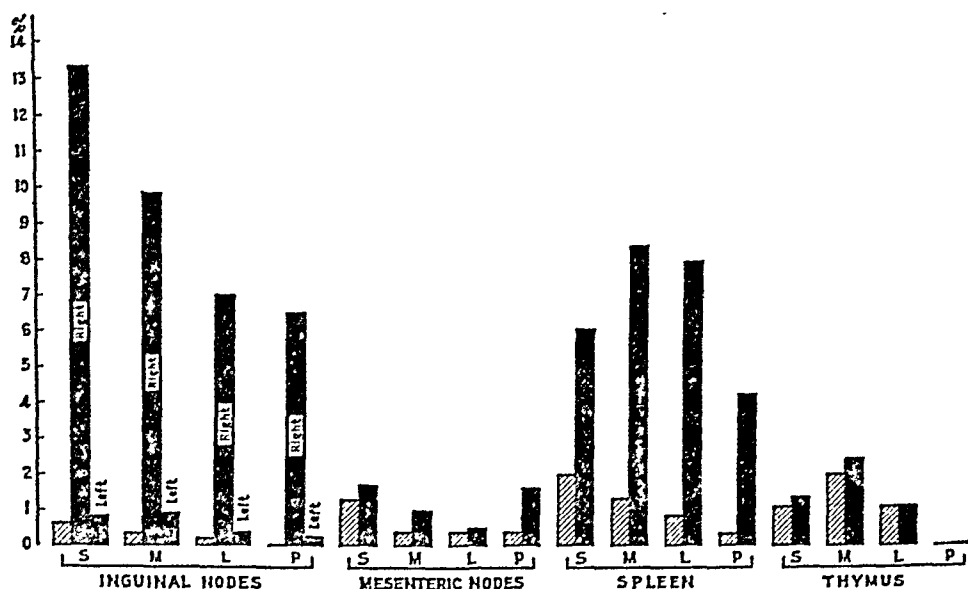


CHART I

A graphic representation of the percentage values of lymphoblasts and plasma cells in lymphoid organs of the normal and infected rats.

Shaded columns = normal animals. Black columns = infected animals. S = small lymphoblasts. M = medium sized lymphoblasts. L = large lymphoblasts. P = plasma cells.

negligible. In addition, microscopic examination of sections of thymus showed only a very slight lymphoblastic hyperplasia; practically all the cortical cells were small cells (Fig. 37). The presence of a large amount of India ink demonstrated that trypanosomes had been actually introduced into the gland (Fig. 36). In contrast, it was of especial interest that in each animal the mediastinal lymph nodes in close proximity to the thymus presented a picture of marked lymphoblastic or plasma cell hyperplasia (Figs. 38 and 39).

TABLE IV

Average Percentage Values of Differential Counts of the Thymus of 7 Normal and 5 Infected Rats: 500 Cells Counted in Each Preparation.

Type of Cell	Normal	Infected
Small lymphoblasts	1.1	2.0
Small immature lymphocytes	83.9 ²	82.7 ²
Small degenerated cells	9.7	9.7
Small lymphocytes	+	0
Medium sized lymphoblasts	2.0	3.0
Medium sized immature lymphocytes, lymphocytes and degenerated cells ¹	0.9	0.6
Large lymphoblasts	1.1	1.2
Large immature lymphocytes, lymphocytes and degenerated cells ¹	0.5	0.1
Plasma cells	+	0.3
Monocyte-like cells	0	0.1
Clasmatocytes	0.4	0.4
Neutrophilic leukocytes	0.1	0.1
Eosinophilic leukocytes	+	0

1. Owing to their scarcity these cells are grouped together under their respective sizes.

2. Includes some cells morphologically slightly different (see text).

+ = present, but less than 0.1 per cent.

DISCUSSION

The characteristic and striking increase of large and medium sized lymphoblasts of the spleen and lymph nodes of rats infected with *Trypanosoma brucei* has again been demonstrated in this experiment. In previous experiments this information was obtained by means of tissue fixed in the usual way, but in the observations here reported the supravital technique was employed.

In this study we find three criteria of considerable value in judging the age of these cells. They are the degree of opacity of the cytoplasm, the number of neutral red bodies, and the number and size of

mitochondria.* Taking these three criteria together we have been able to differentiate the lymphoid cells into three main groups — lymphoblasts, immature lymphocytes and lymphocytes. The possibility of differentiating the small lymphoid cells into these three groups further confirms our previous assumption that failure of some of the small lymphoid cells to hypertrophy is due to the maturity of the cells.

In certain instances, however, a clear-cut classification may not be possible, as in a small cell where scarcity of cytoplasm makes it difficult to estimate its opacity. In such a case the character of the mitochondria and the presence or absence of neutral red bodies are the important points that determine the degree of maturity. On the other hand, increased opacity of the cytoplasm alone is not always dependable for the identification of young cells; for plasma cells (Figs. 18, 25), which are end products as far as further proliferation is concerned, possess cytoplasm as opaque as that of the lymphoblasts. Nor does the presence of neutral red bodies always signify maturity; for lymphoblasts, while in the circulation, or in the presence of some abnormal irritant, can exhibit conspicuous neutral red bodies without loss of the function of proliferation.

Since they represent a series of transitional stages between the typical small lymphoblasts and the typical lymphocytes, the small immature lymphocytes necessarily vary in the degree of maturity. There is no satisfactory evidence to prove that some of the younger forms of this group can hypertrophy like the typical small lymphoblasts, but such a possibility is suggested by the fact that the number of the newly formed large lymphoblasts in the sections of the spleen and lymph nodes examined 48 hours after inoculation is larger than that of the small lymphoblasts normally existing in these organs, as shown in Table II.

The present experiments support the following opinion on the origin of the plasma cells, formed on the basis of the previous histological study in experimental trypanosomiasis. During the terminal phase of the infection, when medium sized and small lymphoblasts are formed in great numbers as a result of repeated and rapid mitotic

* After the completion of the present investigation in 1931 our attention was called to the work of Wiseman² who used the basophilia of the cytoplasm and the mitochondria of the cell as criteria for the determination of the age of the cells in the peripheral blood. It is interesting to note that his and our methods of classification of the lymphoid cells are essentially in agreement.

division of larger lymphoblasts, there is also a rapid and concomitant appearance of the plasma cells. The presence of many transitional forms between typical lymphoblasts and typical plasma cells (Figs. 14, 15, 16, 17, 23, 24, 29, 30 and 31) is very suggestive of the lymphoblastic origin of plasma cells.*

With respect to the thymus, it will be remembered that the origin of the cortical cells is still under discussion. Some investigators believe in their epithelial origin,^{5,6,7} and others derive them from lymphocytes.^{8,9} From the morphological point of view most of the thymic cells in the supravitaly stained films are indistinguishable from lymphoid cells, but some of them contain several conspicuous deep red bodies which are only occasionally seen in the lymphoid cells from other lymphoid organs. This feature, however, does not of itself seem sufficient evidence for the assumption that they are different from lymphoid cells. The morphological differences among the ordinary lymphoid cells themselves may sometimes be even greater. On the other hand, Table II shows 9.7 per cent of degenerated cells in the films from thymus and 21.2 to 29.3 per cent of similar cells in those from other organs. This suggests that there is an actual physiological difference. Contributory evidence on this point is furnished by the failure on the part of the thymic cortical cells to show a hyperplastic reaction after the direct injection of trypanosomes into the organ. The failure to react is not due to a lack of young forms, as differential counts have shown. A similar condition is found in cases of lymphatic leukemia in which all the lymphoid organs, except the thymus, are enlarged.^{6,10} This difference in behavior suggests that the thymic cortical cells are not of lymphoid origin, or, if they are, that conditions prevailing in the thymus successfully prevent them from reacting in the manner of lymphoid cells, as in other lymphoid organs.

* Recently Miller⁴ has demonstrated the primitive connective tissue cells as the origin of plasma cells in rabbits following repeated injections of tuberculo-proteins. He did not specify the time interval between the first injection and autopsy. But if we assume that the injections were made once daily, the average of this interval would be at least 17 days. Since it has been demonstrated in our experiments that lymphoblasts can proliferate and transform into plasma cells in 6 or 7 days, it seems that his observations were made too late to exclude definitely the possibility of lymphoblastic origin of the plasma cells.

SUMMARY

The large, medium sized and small lymphoid cells of the spleen and lymph nodes of normal rats can be classified by means of the supravital technique as lymphoblasts, immature lymphocytes and lymphocytes. The proportions of these different types of cells are found to be altered in the rats infected with *Trypanosoma brucei* by the increase in the percentage of lymphoblasts and plasma cells. The assumption made in the previous report ¹ that the initial appearance of many large lymphoblasts in the lymphoid organs of the infected animals is due to hypertrophy of the small "primitive cells" (lymphoblasts) receives confirmation from the results of the present study. These hypertrophic lymphoblasts produce by mitosis other lymphoblasts, which later transform into plasma cells. The lymphocytes and most of the immature lymphocytes have not been found capable of hypertrophy or mitotic division.

Functionally the cortical cells of thymus are different from the small lymphoid cells of the spleen and lymph nodes.

The classification of the different types of lymphoid cells permits of both qualitative and quantitative studies of cells of lymphoid tissues under normal and pathological conditions.

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DESCRIPTION OF PLATES

PLATE 39

The cells illustrated in this plate were drawn within 10 minutes after the films of the organs were made according to the technique described in the text. They were from the spleen, lymph nodes (inguinal and mesenteric), and thymus of 2 normal and 2 infected rats. Cells from infected animals have their numbers printed in arabic type; those of the normal animals in italics. The size of a red blood cell is included for comparison.

The following types of cells are illustrated:

Small lymphoblasts — 1, 2, 13.

Small immature lymphocyte, younger form — 3.

Small immature lymphocytes, older form — 4, 5, 6.

A type of small immature lymphocytes of thymus — 10 to 12.

Small lymphocytes — 7 to 9.

Transitional forms between small lymphoblasts and typical plasma cells — 14 to 17.

Typical small plasma cell — 18.

Small degenerated cell — 19.

Medium sized lymphoblasts — 20, 22.

Medium sized immature lymphocyte — 21.

Transitional forms between medium sized lymphoblasts and plasma cells — 23, 24.

Medium sized plasma cell — 25, 26.

Large lymphoblasts — 27, 28.

Transitional forms between large lymphoblasts and plasma cells — 29 to 31.

Cells from inguinal lymph nodes of normal rat — 4, 6, 7, 19.

Cells from mesenteric lymph nodes of normal rat — 8, 20.

Cells from spleen of normal rat — 1, 3, 27.

Cells from thymus of normal rat — 5, 28.

Cells from inguinal lymph node of infected rat — 2, 14, 15, 16, 17, 18, 22, 23, 25, 26, 30, 31.

Cell from mesenteric lymph nodes of infected rat — 21.

Cells from spleen of infected rat — 13, 24.

Cell from thymus of infected rat — 11.

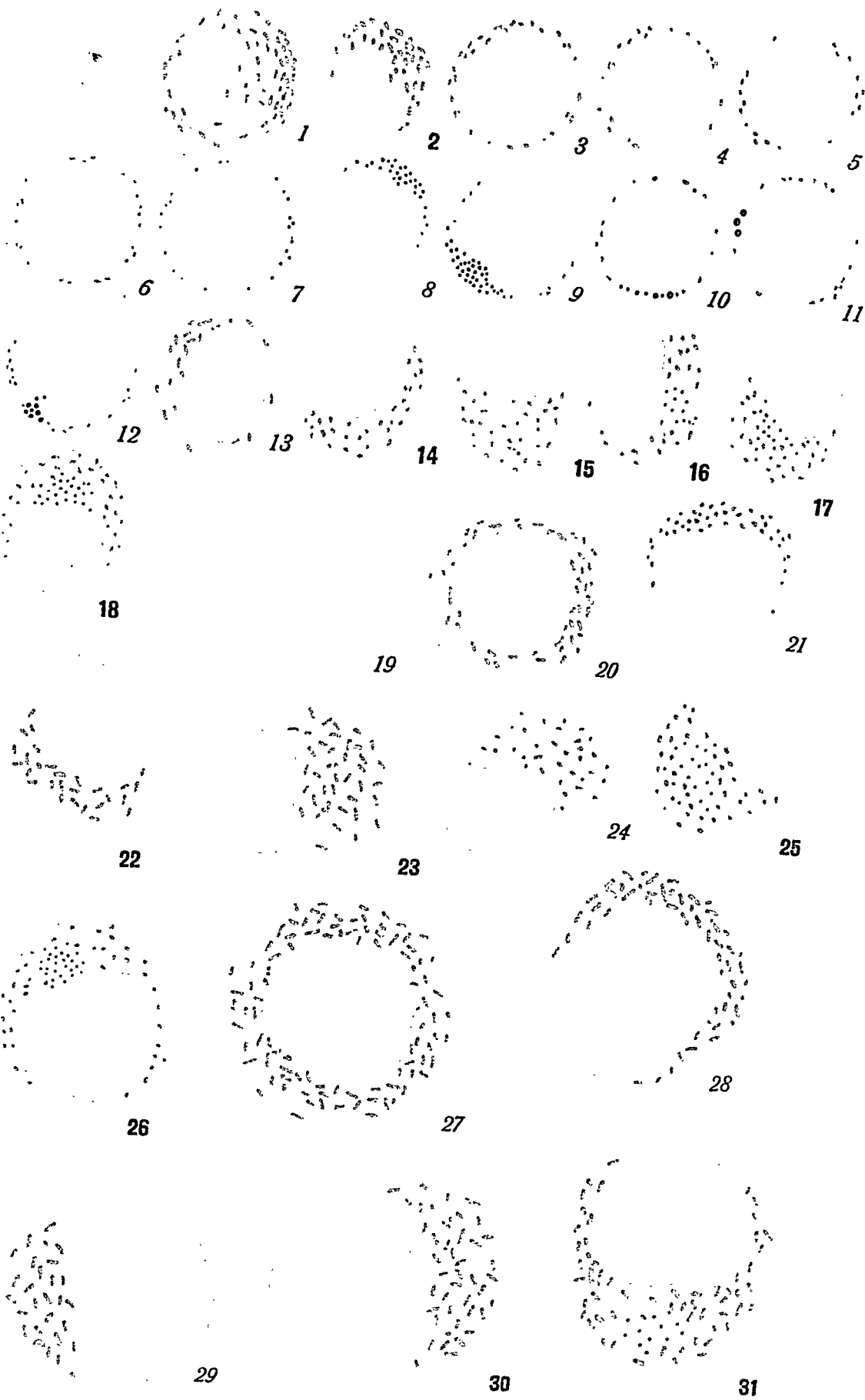
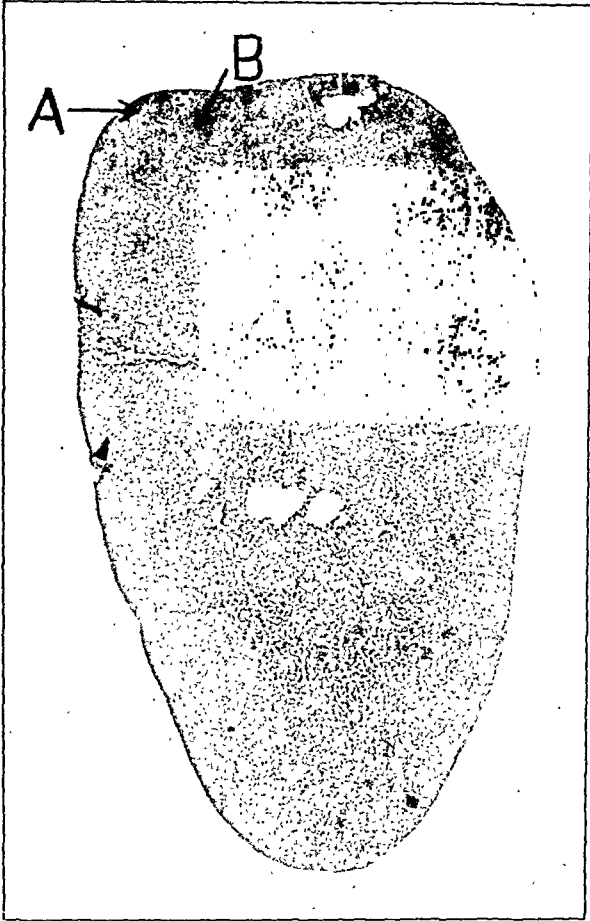
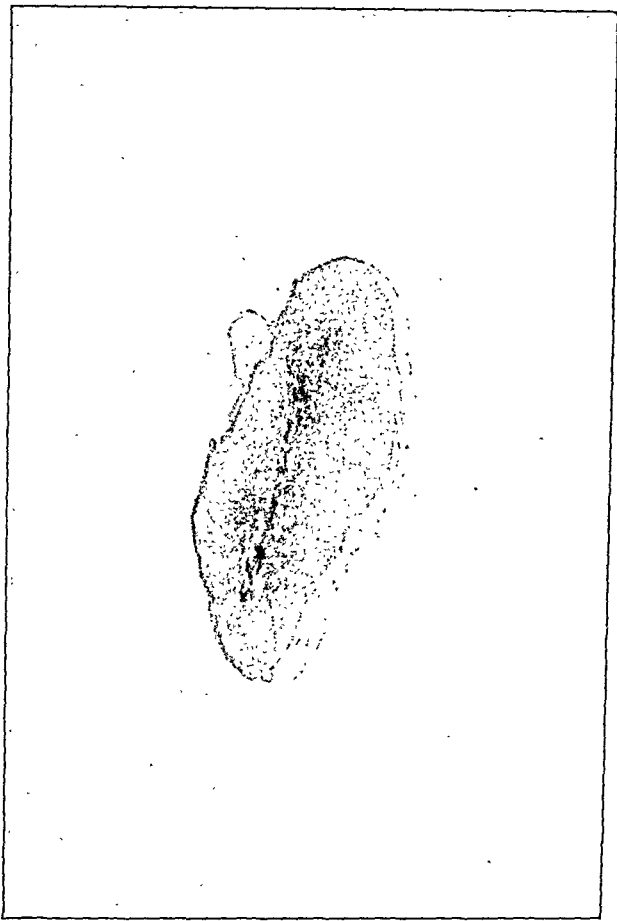


PLATE 40

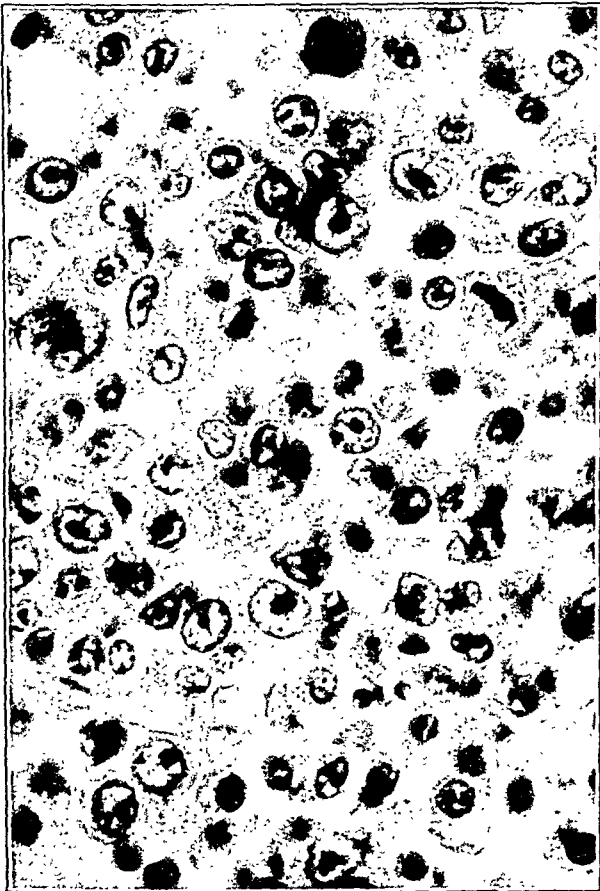
- FIG. 32. Low power view of the right inguinal lymph node of Rat 165, which was injected subcutaneously in the right inguinal region with about 10,000 trypanosomes and killed on the 7th day. $\times 11$.
- FIG. 33. Low power view of the left inguinal lymph node of the same rat (No. 165). It is much smaller than the node shown in Fig. 1. $\times 11$.
- FIG. 34. High power view of the cortical tissue of the right inguinal lymph node in the area marked "A" in Fig. 1. Numerous large lymphoblasts are seen. One cell is undergoing mitosis. Hematoxylin and eosin stain. $\times 800$.
- FIG. 35. High power view of the cortical tissue of the same lymph node in the area marked "B" in Fig. 1, showing lymphoblasts, plasma cells and intermediate forms between the lymphoblasts and plasma cells. Hematoxylin and eosin stain. $\times 800$.



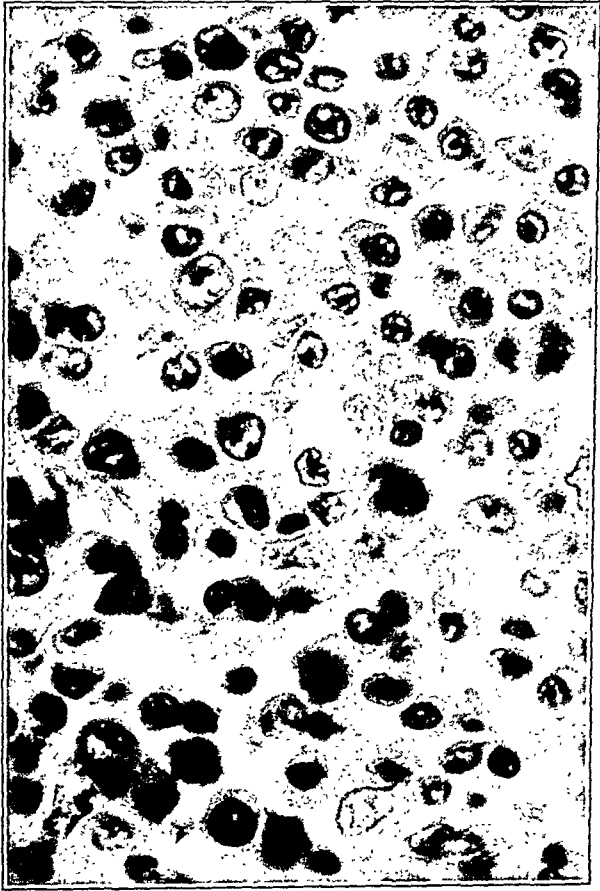
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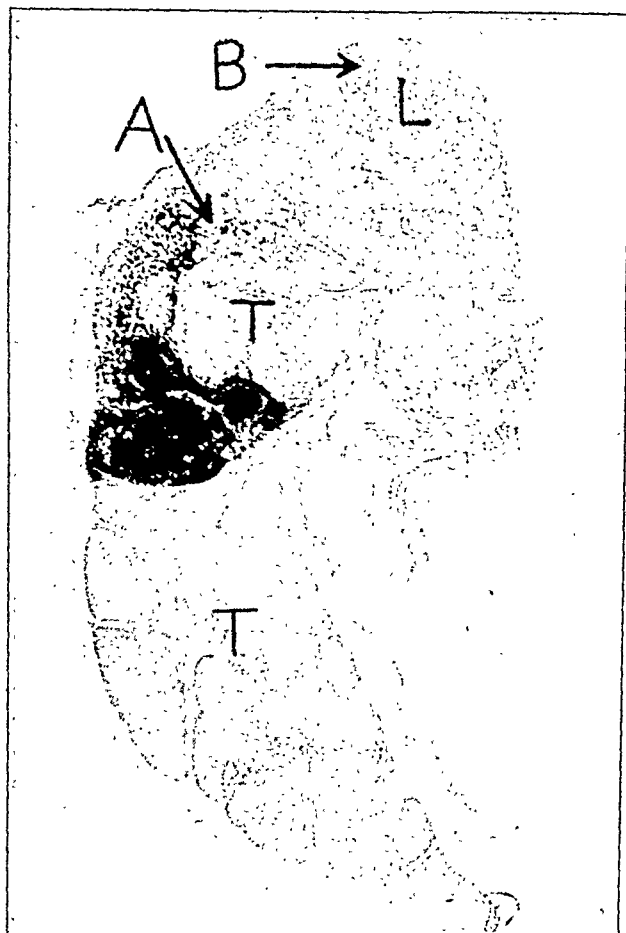
PLATE 41

FIG. 36. Low power view of thymus (T) and mediastinal lymph node (L) of Rat 186. This animal was injected intrathymically with about 3000 trypanosomes mixed with India ink (seen in this illustration as intensely black masses or particles), and was then killed on the 5th day of infection. Section lightly stained with hematoxylin. $\times 9.5$.

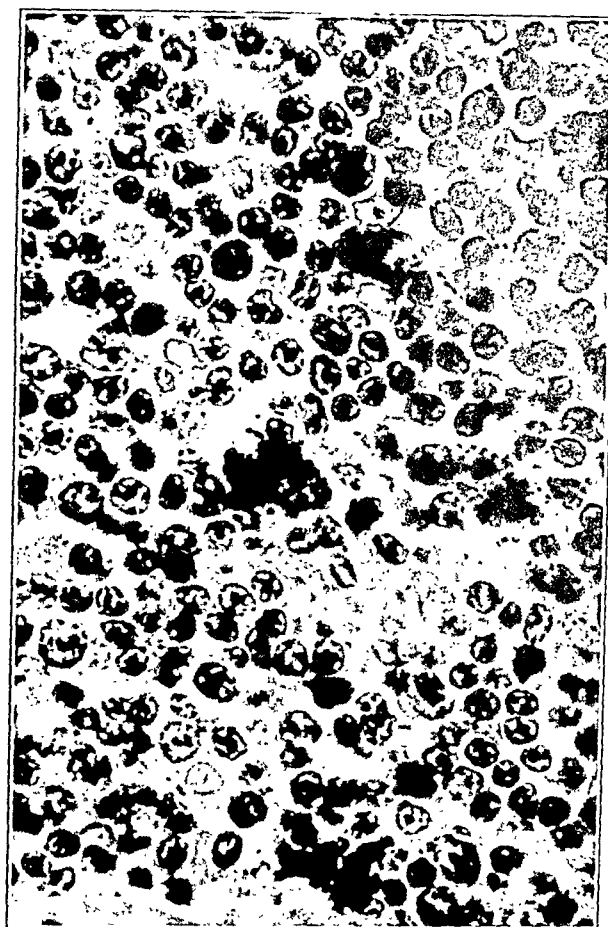
FIG. 37. High power view of an area in the thymic cortex, indicated by "A" in Fig. 5, but from the next serial paraffin section. Masses of ink present in the tissue; no increase of large lymphoblasts seen. Mallory's eosin-methylene blue stain. $\times 800$.

FIG. 38. High power view of an area in the mediastinal lymph node indicated by "B" in Fig. 5, but also from the next serial paraffin section. Many large lymphoblasts are present among the small lymphoid cells. Mallory's eosin-methylene blue stain. $\times 800$.

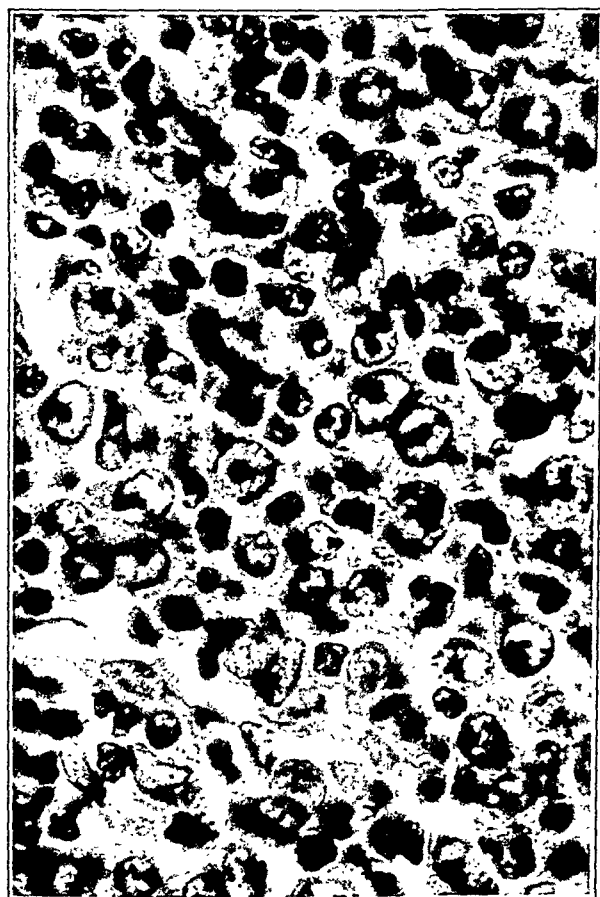
FIG. 39. High power view of an area in the cortex of the mediastinal lymph node of Rat 187, which was injected intrathymically in the same manner as Rat 186 and killed on the 7th day of infection. Numerous plasma cells, and a few lymphoblasts and transitional forms between the plasma cells and lymphoblasts are seen. Hematoxylin and eosin stain. $\times 800$.



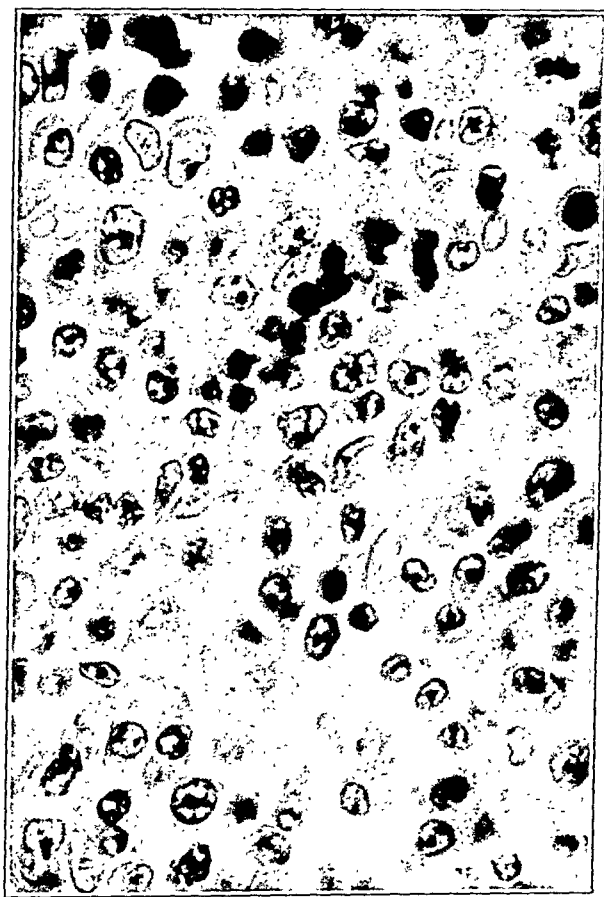
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Hu

Lymphoid Cells of Spleen, Lymph Nodes and Thymus

STUDIES ON THE MATURE AND IMMATURE LYMPHOID CELLS OF THE PERIPHERAL BLOOD OF NORMAL RATS AND RATS INFECTED WITH *TRYPANOSOMA BRUCEI* *

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It has been shown in previous experiments^{1,2} that lymphoblasts of all sizes are present in the diffuse lymphoid tissue of the spleen and lymph nodes of normal white rats, and that in animals infected with *Trypanosoma brucei* the small lymphoblasts in these organs hypertrophy to form large lymphoblasts, which in turn produce other lymphoblasts by mitosis. It has also been shown that in the latter part of the infection many plasma cells appear in the lymphoid organs as a result of direct transformation of the lymphoblasts.

In view of the remarkable lymphatic hyperplasia taking place in the spleen and lymph nodes of the infected rats, and because of the intimate relation between the lymphoid cells of the blood and those of the lymphoid organs, studies using the supravital technique were made on the blood cells of these normal and infected animals.

MATERIAL AND METHODS

Healthy male rats from 4 to 6 months of age, fed on a uniform diet, were used. The infected material consisted of citrated blood of an infected rat, diluted 10 to 20 times with normal saline. After a careful study of the blood of these animals they were each injected intraperitoneally with an amount of the material containing approximately 10,000 parasites.

Since it was necessary to secure perfectly fresh and clean drops of blood for supravital study and for cell counts, the usual methods of bleeding were found to be unsatisfactory. The cutting off the tip of the tail with scissors, or the severing of the tail veins with a razor, involves too much tissue injury and danger of secondary infection, especially when the animal is to be bled repeatedly in the course of the experiment; furthermore, the blood so obtained coagulates quickly and therefore is unsuitable for the purpose of counting. The puncturing of a deeply seated tail vein with a needle is not always successful. The veins of the feet, though easily accessible, are too small. The best are the saphenous veins, which are superficial and large. The skin on the inner surface of the thigh was shaved and thoroughly cleansed with alcohol and ether. The skin over the vein was drawn taut and held immobile so as to reduce the chance of hematoma formation. After the vein was punctured a drop of blood appeared on the skin

* Received for publication May 12, 1933.

surface. The size of the drop could be regulated, to a considerable extent, by the depth of the puncture and by the pressure applied on the vein proximal to the wound. When the desired amount of blood was obtained gentle pressure applied to the wound prevented further loss of blood or the development of a subcutaneous hematoma. By using the left and right veins alternately it was possible to bleed the animals for many days with very little loss of blood and a minimal amount of tissue injury. To prevent struggling, light ether anesthesia was used, which was found to produce no objectionable effect on the animals.

The trypanosomes, which may become very numerous in the blood, take up both Janus green and neutral red stains. Care must be exercised to use the correct quantity of stain so as to avoid overstaining and yet to ensure satisfactory demonstration of the structures of the cells.

OBSERVATIONS ON THE BLOOD OF NORMAL RATS

The non-granular white blood cells of rats show, in supravitaly stained films, great difference in size and opacity of their cytoplasm and in the number, size and arrangement of their mitochondria and neutral red bodies.

In order to evaluate the significance of these differences, a detailed classification such as that shown below is necessary. Code names are used for the sake of convenience.

SA ₀	MA ₀	LA ₀	Deg.
SA ₁	MA ₁	LA ₁	Mono.
SA ₂	MA ₂	LA ₂	Plasma cell
SA ₃	MA ₃	LA ₃	Clasmatocyte
SA ₄	MA ₄	LA ₄	Myelocyte
SA ₅	MA ₅	LA ₅	
SB ₀	MB ₀	LB ₀	
SB ₁	MB ₁	LB ₁	
SB ₂	MB ₂	LB ₂	
SB ₃	MB ₃	LB ₃	
SB ₄	MB ₄	LB ₄	
SB ₅	MB ₅	LB ₅	
SC ₁	MC ₁	LC ₁	
SC ₂	MC ₂	LC ₂	
SC ₃	MC ₃	LC ₃	
SC ₄	MC ₄	LC ₄	
SC ₅	MC ₅	LC ₅	

EXPLANATION

- S = small (Figs. 1-37).
M = medium sized (Figs. 38-56).
L = large (Figs. 57-86).
A = lymphoblasts, characterized by opaque cytoplasm and coarse, abundant mitochondria (Figs. 1-9, 38-41, 57-65).
B = immature lymphocytes, representing the intermediate forms between lymphoblasts and lymphocytes, having less opaque cytoplasm; neutral red bodies generally present; mitochondria less coarse and less abundant than in the lymphoblasts (Figs. 10-20, 42-48, 67-75).

C = lymphocytes, characterized by clear cytoplasm and fine scanty mitochondria; neutral red bodies practically always present (Figs. 21-37, 49-56, 76-86).

o = neutral red bodies absent (Figs. 1, 2, 38, 57, 58).

1 = few or numerous neutral red bodies diffusely arranged without any definite pattern; nucleus rounded or slightly irregular (Figs. 3-14, 19, 21-24, 29, 39, 42, 43, 49, 50, 59-62, 67-70, 76).

2 = neutral red bodies tend to concentrate in one part of the cell, usually in the slight concavity of the nucleus (Figs. 15-18, 26-28, 30, 40, 41, 44, 51-54, 63, 64, 77, 79).

3 = neutral red bodies concentrated in the indentation of the nucleus; nuclear indentation deeper (Figs. 31, 45).

4 = nucleus shows exaggerated indentation, so that it becomes V- or U-shaped, with the deep concavity filled by neutral red bodies (Figs. 32-35, 46, 47, 55, 56, 71-73).

5 = nucleus shows multiple indentations or folds, so that it may become actually lobulated. Neutral red bodies are distributed especially in the indentations or in the folds (Figs. 36, 37, 48, 74, 75, 78, 80-85).

Deg. = degenerated cells, each having a round, homogeneous, usually small nucleus, with unstained small vacuoles or granules usually situated on one side of it. Cytoplasm edematous or so swollen as to become invisible (Fig. 25).

Mono. = monocyte-like cells with kidney-shaped nuclei and rosettes of neutral red bodies. The term "monocyte-like" is used here to indicate cells having the above-mentioned characteristics, without implying that they belong to a cell strain different from that of the lymphoid cells (Figs. 87-90).

PMN = polymorphonuclear neutrophilic leukocytes.

PME = polymorphonuclear eosinophilic leukocytes.

In accordance with the above classification a total of 1000 white blood cells from twenty normal rats was counted each day on 6 different days. As the counts are fairly constant from day to day only the averages are shown in Table I.

The important points shown in this table are as follows. (1) In the peripheral blood of normal rats there are not only large lymphoblasts (total LA, 1.33 per cent) and medium sized lymphoblasts (total MA, 0.9 per cent), but also small lymphoblasts (total SA, 0.85 per cent). (2) Most of the blood lymphoblasts, in contrast to the lymphoblasts of the spleen and the lymph nodes, contain neutral red bodies. (3) There is a higher percentage of small lymphocytes in the peripheral blood (total SC, 23.11 per cent) than in the lymphoid organs (0.9 to 1.9 per cent), as shown in Table II of the preceding report.²

OBSERVATIONS ON THE BLOOD OF INFECTED RATS

Red Blood Cells and Hemoglobin: In another set of 10 rats the number of erythrocytes and the percentage of hemoglobin were determined the day before, and then 7 days after they became

infected. A moderate anemia was found as a result of the infection (Table II).

Parasites and White Blood Cells: For the study of parasites and the white blood cells during infection we used the same group of 20 rats, whose normal white counts are shown in Table I. Two days after the last normal count was made each of these rats was inoculated intra-

TABLE I

Average Percentage Values of 6 Differential Counts (Done on April 2, 3, 4, 6, 7 and 9, 1931 Respectively) of the White Blood Cells of 20 Normal White Rats (Nos. 131 to 150)

SA ₀	0.03	MA ₀	0.10	LA ₀	0.17	Deg.	0.15
SA ₁	0.82	MA ₁	0.73	LA ₁	1.08	Mono.	2.33
SA ₂	0.00	MA ₂	0.02	LA ₂	0.03	Plasma cell	0.02
SA ₃	0.00	MA ₃	0.03	LA ₃	0.05	Clasmatocyte	0.02
SA ₄	0.00	MA ₄	0.00	LA ₄	0.00	Myelocyte	0.07
SA ₅	0.00	MA ₅	0.02	LA ₅	0.00	PMN.	26.58
<hr/>		<hr/>		<hr/>		PME.	1.70
Total SA	0.85	Total MA	0.90	Total LA	1.33		
SB ₀	0.83	MB ₀	0.00	LB ₀	0.00		
SB ₁	32.42	MB ₁	0.50	LB ₁	0.22		
SB ₂	0.20	MB ₂	0.07	LB ₂	0.10		
SB ₃	0.97	MB ₃	0.63	LB ₃	0.80		
SB ₄	0.23	MB ₄	0.62	LB ₄	0.65		
SB ₅	0.23	MB ₅	0.52	LB ₅	0.88		
<hr/>		<hr/>		<hr/>			
Total SB	34.88	Total MB	2.34	Total LB	2.65		
SC ₁	17.35	MC ₁	0.87	LC ₁	0.43		
SC ₂	1.62	MC ₂	0.27	LC ₂	0.10		
SC ₃	3.40	MC ₃	0.30	LC ₃	0.12		
SC ₄	0.16	MC ₄	0.13	LC ₄	0.17		
SC ₅	0.58	MC ₅	0.33	LC ₅	0.35		
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Total SC	23.11	Total MC	1.90	Total LC	1.17		

peritoneally with approximately 10,000 parasites. Daily counts were made of parasites beginning the 1st day, and of white blood cells beginning the 2nd day, after inoculation. Table III shows the number of parasites in the blood of these animals from the time of injection to the time of death.

From this table it will be seen that the animals dying early happen to be smaller and the number of parasites in their blood shows a rapid rise reaching its peak with the death of the animal; the animals that survived longer, on the other hand, happen to be larger and the number of parasites shows a gradual rise, then a rapid fall, and finally

TABLE II

The Red Blood Cell Counts and Hemoglobin Values of 10 Rats Before, and 7 Days After, Intraperitoneal Injection of Approximately 10,000 Parasites

Rat No.	Before infection		On the 7th day of infection	
	Red blood cells per cmm.	Hemoglobin per cent ¹	Red blood cells per cmm.	Hemoglobin per cent
102.....	10,790,000	100	5,730,000	65
103.....	9,770,000	88	7,010,000	73
104.....	9,410,000	92	6,430,000	64
105.....	11,110,000	100	7,770,000	78
106.....	9,790,000	89	7,360,000	63
107.....	10,620,000	100	7,360,000	65
108.....	10,030,000	94	6,870,000	70
109.....	9,730,000	88	7,170,000	70
110.....	10,140,000	91	7,140,000	73
111.....	9,300,000	92	7,940,000	78
Average	10,069,000	93.4	7,078,000	69.9

1. 100 per cent = 17 gm. of hemoglobin.

TABLE III

Daily Counts of Parasites in the Blood of 20 Infected White Rats

Group *	Rat No.	Body wt. gm.	Survival period days	Day after inoculation								
				1st	2nd	3rd	4th	5th	6th	7th	8th	9th
				Number of parasites in thousands per cmm.								
A	145	234	5	0	+	6	154	500
A	140	251	5	0	0	5	97	475
A	143	342	5	0	+	5	155	467
A	142	211	6	0	+	7	231	443	430
A	144	304	6	0	0	3	56	385	***
A	141	357	6	0	0	2	101	316	348
A	135	350	6	0	+	3	47	205	526
A	139	307	6	0	0	6	106	290	319
**	136	360	7	0	0	2	62	262	364	392
**	150	407	7	0	+	4	125	267	352	338
B	131	440	9	0	+	2	46	116	3	10	195	***
B	147	379	9	0	0	5	91	79	2	20	252	***
B	146	392	9	0	+	5	55	56	1	6	213	587
B	149	335	9	0	+	+	39	99	1	7	194	556
B	133	377	9	0	+	2	56	+	2	6	136	410
B	148	357	9	0	+	3	52	+	1	17	192	560
B	137	320	9	0	+	5	13	0	3	12	164	513
B	134	392	9	0	+	6	60	4	+	14	96	290
B	138	315	9	0	0	2	21	0	1	9	97	327
B	132	430	9	0	+	7	34	155	4	8	300	524

* See text.

** Not included in either Group A or Group B.

*** Animal died before counts could be made.

+ = less than 1000 per cmm.

TABLE IV

Average Percentage Values of the White Blood Cells Obtained from the Counts Made on 8 Rats (Group A) During Infection, with their Normal Counts as Control

Code name	Normal *	Day after inoculation				
		2nd	3rd	4th	5th	6th**
SA ₀	0.08	0	0	0.25	2.5	0
SA ₁	1.25	1.5	2.0	1.0	5.5	3.5
SA ₃	0	0	0	0	0.25	0
Total SA	1.33	1.5	2.0	1.25	8.25	3.5
SB ₀	0.79	1.25	2.0	0.25	1.25	0.5
SB ₁	33.33	25.5	19.5	24.5	21.0	27.0
SB ₂	0.13	0.5	0	0	0.25	0
SB ₃	0.79	0	0.75	0.25	0	0
SB ₄	0.17	0	0	0	0	0
SB ₅	0.29	0.25	0.25	0	0	0
Total SB	35.5	27.5	22.5	25.0	22.5	27.5
SC ₁	15.49	18.5	12.0	6.75	7.25	11.0
SC ₂	2.04	1.75	0.75	0.25	1.0	2.0
SC ₃	3.71	2.0	1.5	0.25	1.0	1.5
SC ₄	0.21	0.25	0	0	0	0
SC ₅	0.54	0.25	0.5	0.5	0	0
Total SC	21.99	22.75	14.75	7.75	9.25	14.5
MA ₀	0.12	0.25	0	0.75	2.5	0
MA ₁	0.46	0.25	2.0	0.5	3.75	2.0
MA ₂	0.04	0	0	0	0	0.5
MA ₃	0.04	0	0	0	0	0.5
MA ₅	0.04	0	0	0	0	0.5
Total MA	0.7	0.5	2.0	1.25	6.25	3.5
MB ₁	0.58	0.75	0.25	0.5	1.5	2.0
MB ₂	0.04	0	0	0	0	0.5
MB ₃	0.50	0.25	1.0	0.25	1.0	0.5
MB ₄	0.62	0	0.25	0	0	0
MB ₅	0.38	0.25	1.5	0.75	1.0	1.0
Total MB	2.12	1.25	3.0	1.5	3.5	4.0
MC ₁	1.08	3.0	1.75	1.25	0	0.5
MC ₂	0.25	0.25	0.25	0	0.5	0
MC ₃	0.33	0.5	0	0	0	0
MC ₄	0.21	0	0.25	0.25	0	0
MC ₅	0.5	1.0	0.5	0	1.0	0
Total MC	2.37	4.75	2.75	1.5	1.5	0.5

TABLE IV (Continued)

Code name	Normal *	Day after inoculation				
		2nd	3rd	4th	5th	6th**
LA ₀	0.08	0.25	0.25	1.0	2.0	1.0
LA ₁	0.58	0.25	1.0	1.75	2.75	1.0
LA ₂	0.04	0.25	0	0	0	0
LA ₃	0.04	0	0.25	0	0.25	0.5
LA ₅	0	0	0.25	0	0	0
Total LA	0.74	0.75	1.75	2.75	5.0	2.5
LB ₁	0.21	0.25	0	1.25	2.0	0
LB ₂	0.08	0	0	0.75	0.75	0
LB ₃	0.79	0.25	1.5	0.25	1.75	0
LB ₄	0.58	0.5	0.75	0	1.25	2.0
LB ₅	0.75	1.0	1.5	0.75	5.75	3.0
Total LB	2.41	2.0	3.75	3.0	11.5	5.0
LC ₁	0.54	1.75	1.5	1.0	0.75	1.5
LC ₂	0.08	0.25	0.25	0.25	0	0
LC ₃	0.13	0.25	0.25	0	0.25	0.5
LC ₄	0.17	0.5	0	0	0	0
LC ₅	0.41	1.25	1.0	1.0	1.0	1.5
Total LC	1.33	4.0	3.0	2.25	2.0	3.5
Deg.	0.04	0.5	0	2.25	0.5	1.5
Mono.	1.87	2.75	3.5	1.25	3.25	6.0
Plasma	0	0	0	0	0	0
Clas.	0.04	0	0	0	0.5	1.0
Myelo.	0.04	0	0.25	0	0	0
PMN	27.83	30.5	37.25	47.5	24.0	25.0
PME	1.63	1.25	3.5	2.75	2.0	2.0
Average total WBC per cmm.	8110	8150	7790	4825	6710	8970

* These values are incorporated in Table I.

** Counts from only 4 animals.

a sharp rise shortly before death. The survival period of the animals and the rate of increase of parasites vary, therefore, according to the intensity of infection, influenced by the size of the animals used. But as this finding has no direct bearing on the problem on hand no further discussion will be made.

In view of the wide variation in the survival period of these animals it is apparent that an average of the white counts of all of them

will be of very little value. These counts are therefore divided into two groups. In Group A are placed the counts of 8 rats dying on the 5th or 6th day of infection, and in Group B those of 10 rats dying on the 8th or 9th day. The counts of 2 animals (Nos. 136 and 150) which died on the 7th day cannot be conveniently placed in either group and are therefore discarded.

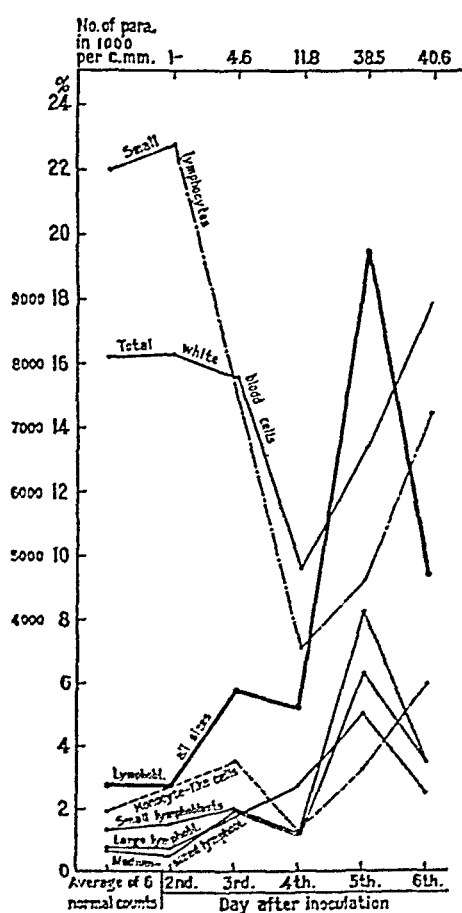


CHART 1

A graphic representation of the important data in Table IV. The differential counts are recorded in percentages; the white blood cell counts in total numbers per cmm.

The average values of the counts in Group A are shown in Table IV and Chart 1; those in Group B in Table V and Chart 2.

The important points revealed by Table IV are as follows. (1) A transient leukopenia, most pronounced on the 4th day of infection, due largely to the reduction in number of small lymphocytes. (2) A marked increase in number of lymphoblasts of all sizes and a similar increase of the medium sized and large immature lympho-

TABLE V

Average Percentage Values of the White Blood Cells Obtained from the Counts of 10 Rats (Group B) during Infection, with their Normal Counts as Control

Code name	Nor- mal*	Day after inoculation							
		2nd	3rd	4th	5th	6th	7th	8th	9th**
SA ₀	0	0	0	0	1.4	0.4	0.2	0.4	0.25
SA ₁	0.63	1.8	1.0	2.6	6.0	1.8	3.2	0.4	2.0
SA ₃	0.03	0	0	0	0.6	0	0.2	0	0
Total SA	0.66	1.8	1.0	2.6	8.0	2.2	3.6	0.8	2.25
SB ₀	0.83	3.2	1.2	0.4	1.8	0.8	1.4	0.8	0.5
SB ₁	33.13	31.6	25.8	21.6	28.4	29.4	31.0	26.6	22.5
SB ₂	0.27	0	0	0.2	0.4	0	0.2	0.4	0
SB ₃	1.2	1.0	1.8	0	0.2	0.2	1.6	0.6	0.25
SB ₄	0.17	0.2	0.4	0	0	0	0.2	0	0.25
SB ₅	0.2	0.2	1.0	0.2	0	0.2	0.6	0.6	0
Total SB	35.8	36.2	30.2	22.4	30.8	30.6	35.0	29.0	23.5
SC ₁	19.03	16.2	11.6	9.6	11.8	21.2	17.8	13.2	8.0
SC ₂	1.3	2.0	0	1.0	0.6	1.2	2.0	1.0	0.75
SC ₃	3.23	2.0	2.4	0.2	0.2	1.4	3.2	3.0	4.5
SC ₄	0.17	0	0.2	0	0	0.2	0	0	0.25
SC ₅	0.47	0.4	0.6	0.2	0	0	0.6	0.6	0
Total SC	24.2	20.6	14.8	11.0	12.6	24.0	23.6	17.8	13.5
MA ₀	0.1	0	0	0	1.8	0.4	0	0.6	1.0
MA ₁	0.77	0	0.6	0.8	4.6	1.6	1.4	1.	1.25
MA ₂	0	0	0	0	0.2	0	0.2	0	0
MA ₃	0	0	0	0.2	0	0	0	0.2	0
MA ₅	0	0	0.2	0	0	0.2	0.6	0	0.25
Total MA	0.87	0	0.8	1.0	6.6	2.2	2.2	1.8	2.5
MB ₁	0.33	0.6	0.6	0.8	0.8	2.0	0.6	1.6	0.25
MB ₂	0.07	0.2	0	0	0.2	0.2	0.2	0	0.25
MB ₃	0.8	1.2	0.8	0.6	0.2	0.6	1.0	1.6	0.25
MB ₄	0.53	1.4	0.2	0	0.4	0.2	0.6	1.0	0.25
MB ₅	0.67	1.0	1.0	0.4	0.6	0.6	1.6	1.2	1.75
Total MB	2.4	4.4	2.6	1.8	2.2	3.6	4.0	5.4	2.75
MC ₁	0.77	2.2	0.2	0.2	0.2	1.6	2.6	2.0	0.5
MC ₂	0.2	0.4	0	0	0	0.6	0.6	0	0.25
MC ₃	0.2	0.6	0.6	0.2	0	0	0.4	0.6	0.25
MC ₄	0.1	0.2	0	0	0	0	0.4	0.4	0.25
MC ₅	0.23	0.2	0.6	0	0	0.2	0.2	0	0.75
Total MC	1.5	3.6	1.4	0.4	0.2	2.4	4.2	3.0	2.0

TABLE V (Continued)

Code name	Nor- mal*	Day after inoculation							
		2nd	3rd	4th	5th	6th	7th	8th	9th**
LAo	0.2	0.2	0	1.2	2.4	0.8	0.4	0.4	0.75
LA1	1.23	0.8	1.4	1.4	2.8	1.4	1.0	1.0	1.0
LA2	0.03	0	0	0.2	0	0	0	0	0
LA3	0	0.2	0.4	0.2	0.4	0.4	0.4	0	0
LA5	0	0	0.2	0	0.4	0	0	0.4	0.25
Total LA	1.46	1.2	2.0	3.0	6.0	2.6	1.8	1.8	2.0
LB1	0.2	0.2	0.2	0.8	1.0	0.8	0.2	0.6	0.5
LB2	0.13	0	0.2	0.2	0.2	0	0.4	0.2	0.25
LB3	0.83	0.2	1.4	1.4	2.4	0.6	0	1.0	0.75
LB4	0.53	0	1.2	0.2	0	1.4	0.4	0.2	1.25
LB5	0.83	0.4	1.6	1.2	3.0	1.0	1.4	2.4	3.5
Total LB	2.52	0.8	4.6	3.8	6.6	3.8	2.4	4.4	6.25
LC1	0.43	0.6	0.2	0	0.4	1.0	0.4	1.2	1.75
LC2	0.07	0	0	0.2	0	0.2	0.4	0	0.25
LC3	0.07	0.2	0	0	0	0	0.2	0.2	0.25
LC4	0.17	0	0	0	0	0	0	0.2	0
LC5	0.2	0.2	0.8	0.4	0.8	1.6	0.8	0.4	1.0
Total LC	0.94	1.0	1.0	0.6	1.2	2.8	1.8	2.0	3.25
Deg.	0.02	3.0	1.2	5.0	7.2	7.6	2.4	2.0	1.25
Mono.	2.30	1.6	1.8	2.4	3.2	5.8	3.6	4.4	5.5
Plasma	0	0	0	0	0	0	0	0	0.25
Clas.	0	0	0	0	0.2	0	0.2	0	0
Myelo.	0.47	0	0	0	0	0	0	0	0
PMN	25.33	23.8	35.8	44.0	14.2	11.0	12.4	24.2	29.5
PME	1.3	2.0	2.8	2.0	1.0	1.4	2.8	3.4	5.5
Average total WBC per cmm.	8610	8140	6950	5730	8100	8450	9170	7180	6680

* These values are incorporated in Table I.

** Counts from only 8 animals.

number and to show increased physical activity. It seems desirable, therefore, to classify these subvarieties simply into two main groups, the typical and the atypical. To the former belong cell Types 0, 1 and 2 and to the latter 3, 4 and 5.

In regard to the cells having the appearance of monocytes we are unable to say whether they are true monocytes or if they merely represent another variation of the atypical lymphoid cells. It is quite definite, however, that they can be traced easily to the atypical

cytes (especially LB5) on the 5th day. (3) An increase in number of monocyte-like cells at the end of infection. These points are graphically shown in Chart 1.

Table V and Chart 2 show the following additional features. (1) The number of small lymphocytes, which shows a sharp fall on the 4th day of infection, as in Table IV, rises to its normal level on the 6th day, but again falls to a lower level at the time of death. (2) The number of lymphoblasts, after a rise on the 5th day, later also falls, but not below the normal level. (3) A few plasma cells appear in the blood at the end of infection.

It was noted that in the different types of lymphoid cells and in the monocyte-like cells the neutral red bodies tend to increase both in number and in size in the latter part of infection. These cells (Figs. 29, 32, 33, 79-86), which are usually large, are often very active. The shape of these cells and their nuclei changes rapidly and the enlarged neutral red bodies are constantly in motion, so that the same cell may appear quite differently at different times (Figs. 79, 80).

It may also be mentioned that the lymphoblasts, usually the large variety, are occasionally seen undergoing mitosis (Fig. 65).

DISCUSSION

In connection with the results obtained in this and the previous studies,^{1,2} three problems are to be considered: the classification of the non-granular white blood cells of the rat, the relation between the tissue lymphoid cells and the blood lymphoid cells, and the proliferative power of what are generally known as the small lymphocytes of the blood.

At the beginning of this report an elaborate classification of the non-granular white blood cells of the rat was given. Examination of the data in this experiment indicates, however, that although it is necessary to separate the lymphoid cells according to their age and size, the classification of each type into six subvarieties (Types 0, 1, 2, 3, 4 and 5) is not essential. Attention is called to the fact that these subvarieties actually form a continuous series of cells and that the morphology of some of them is by no means constant, but is apt to change from one type to another (Figs. 79, 80). On the other hand, during infection some of these subvarieties, especially Type 5 of the medium sized and the large cells, tend to increase in

lymphoid cells and, like the latter, are also increased in number in this infection.

We may now present our classification of the non-granular white blood cells of the rat as follows:

1. Lymphoblasts	small,	medium sized,	large.
2. Atypical lymphoblasts	"	"	"
3. Immature lymphocytes	"	"	"
4. Atypical immature lymphocytes ...	"	"	"
5. Lymphocytes	"	"	"
6. Atypical lymphocytes	"	"	"
7. Degenerated lymphoid cells			
8. Monocyte-like cells			
9. Plasma cells			
10. Clasmatocytes			

In regard to the relation between the lymphoid cells of the tissue and the blood it is first of all important to know whether the increased number of lymphoblasts in the blood during infection is due to an increased emigration from the spleen and lymph nodes, or whether it is due to direct proliferation of the circulating lymphoblasts. It is probable that lymphoblasts without neutral red bodies are emigrated cells, since most of the lymphoblasts in the spleen and lymph nodes are of this type. But emigration of lymphoblasts does not seem to take place readily for, although many large lymphoblasts appear in the spleen as early as 48 hours after the animals become infected,¹ the blood does not show any increase of such cells until 2 or 3 days later, when many parasites appear in the circulation. Furthermore, when the medium sized lymphoblasts are formed in great numbers in the lymphoid organs in the latter part of infection there is no corresponding increase of such cells in the blood. On the other hand, the repeated finding of mitoses among the circulating lymphoblasts is a further evidence of the local origin of some of these cells.

In view of the great severity of the infection and the presence in the blood stream of tremendous numbers of parasites toward the end of the disease the reduction in number of blood lymphoblasts at this time, as shown in Table V, needs to be explained. It will be recalled in this connection that at this stage of the disease sections of these organs, especially the spleen, also showed a definite reduction in number of lymphoblasts. We are inclined to think that it is due either to excessive cell destruction, or to exhaustion of the prolifera-

tive power of the lymphoblasts in the presence of an overwhelming infection.

The presence of a higher percentage of small lymphocytes in the blood than in the lymphoid organs suggests either one or both of the following two possibilities: that the small lymphocytes of the organs

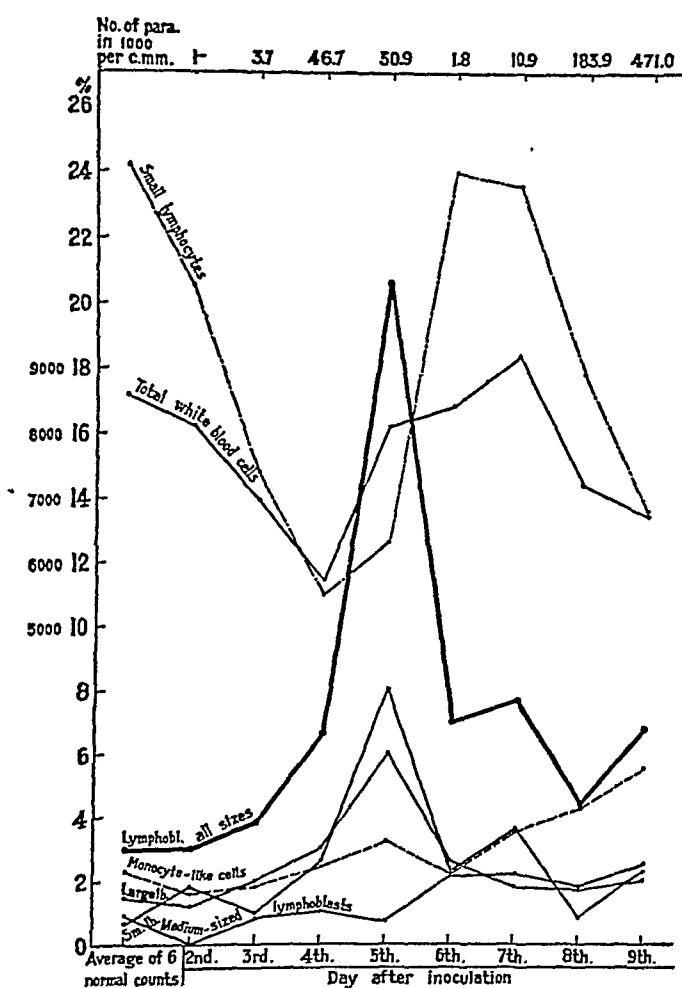


CHART 2

A graphic representation of the important data in Table V. The differential counts are recorded in percentages; the white blood cells in total numbers per cmm.

emigrate more readily than the immature lymphocytes, or that the latter cells mature more quickly in circulation than in the tissue. The marked reduction in small lymphocytes, which is largely responsible for the initial leukopenia in the infected animals, is interesting and can probably be explained by the fact that since they are older they are more susceptible to injury than the others.

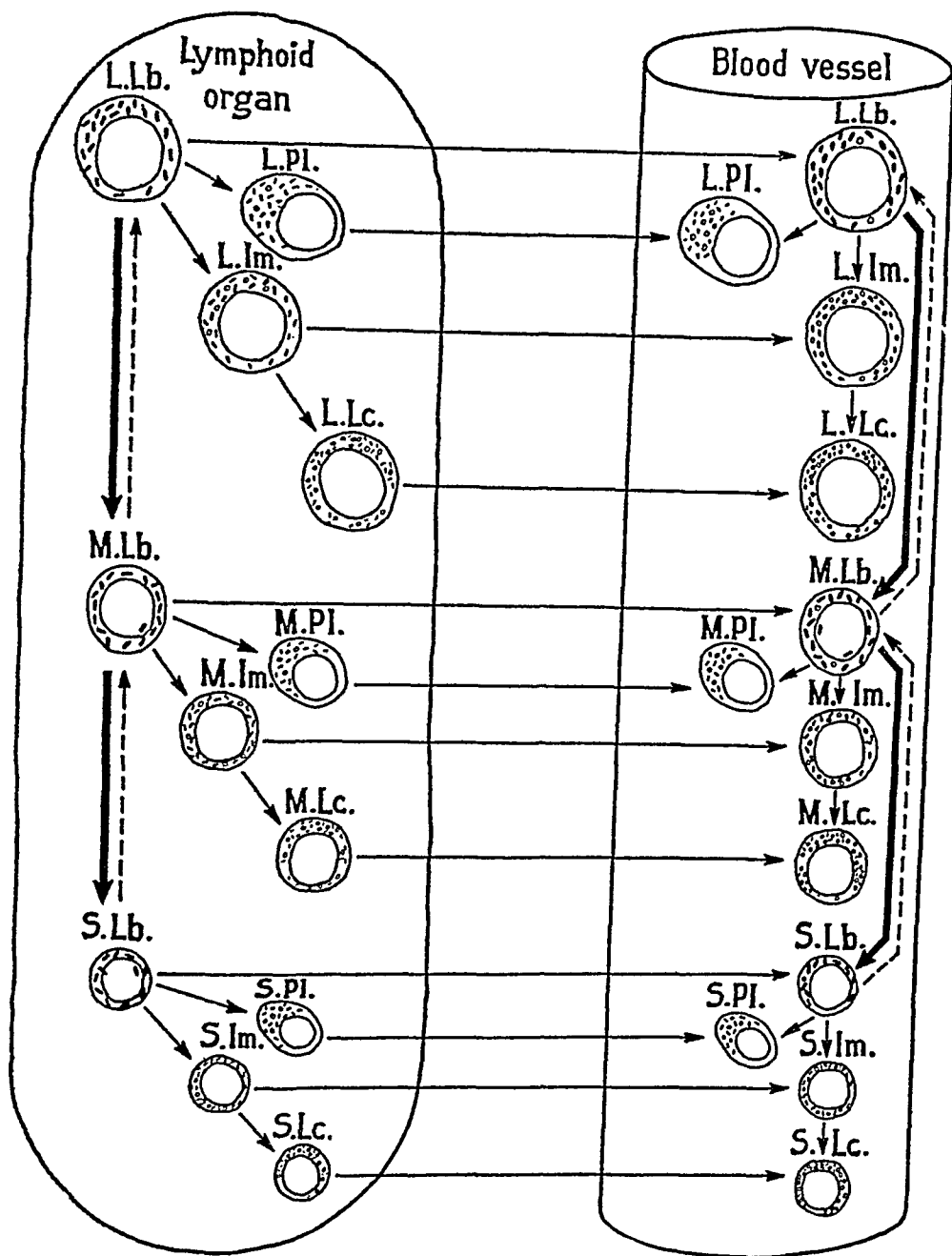
Although many plasma cells are produced in the tissues by the transformation of the lymphoblasts, very few such cells are seen in the circulation. Like the lymphoblasts they do not readily pass into the blood stream. On the other hand, it is still possible that some of the circulating lymphoblasts may be transformed into plasma cells, since they are morphologically identical with the tissue lymphoblasts, whose ability for such a transformation seems to be quite definite.

Text-figure 1 represents the relation between the different types of tissue and blood lymphoid cells.

Although what is generally called the small lymphocyte of the blood has been studied by many investigators, its potentialities for hypertrophy, proliferation and transformation are still a subject of dispute. Representing probably the more general view is Naegeli,³ who considers that all the small lymphoid cells of the blood are true mature lymphocytes, incapable of further growth or transformation. He believes, however, that the small lymphoid cells of the lymphoid organs are young cells, capable of forming large lymphoblasts by hypertrophy. The blood lymphocytes, according to him, are derived from these lymphoblasts.

Cunningham, Sabin, and Doan⁴ also believe that the so-called small lymphocytes of the blood are more mature than the other cells of the lymphoid series. But as mitoses have been observed in these cells, they placed them in their schema on the same level with myelocytes, and not with polymorphonuclear leukocytes of the granular series. They differ from Naegeli, who holds that the size of the cell has nothing to do with its maturity, and look upon the large lymphocytes as being younger than the small lymphocytes. They derive lymphocytes from lymphoblasts and the latter from primitive cells normally present in lymphoid organs.

An entirely different view is expressed by the representatives of the unitarian theory championed especially by Maximow.⁵⁻⁹ He believes that there is no difference between the lymphocytes of the blood and those of the tissue, and that the small lymphocytes of the blood are, like the larger lymphocytes, potentially capable of unrestricted development, even into cells other than those of the lymphoid series. In culture and in ligated blood vessels he has observed hypertrophy of the small lymphocytes of the blood and their eventual transformation into many other types of cells — monocytes, epithelioid cells, fibroblasts.



→ = cell division → = hypertrophy → = transformation or emigration
 L = large M = medium sized
 S = small Lb = lymphoblast Im = immature lymphocyte
 Lc = lymphocyte Pi = plasma cell Mitochondria represented by black dots. Neutral red bodies represented by circles.

TEXT-FIG. 1

A representation of the relation between the different types of tissue and blood lymphoid cells

We are inclined to think that the conflicting views of Naegeli and Maximow are based upon observations made under imperfect experimental conditions. For a satisfactory study of the development of lymphoid cells it is necessary to have not only a state of marked lymphoblastic hyperplasia, which enables the observer to follow closely the rapid changes in the lymphoid cells, but also a technique that makes possible the differentiation of cells which, in fixed smears or tissues, have an identical appearance. Separation of the blood lymphoid cells into lymphoblasts, immature lymphocytes and lymphocytes* offers an easy explanation to the above-mentioned conflicting views. It is apparently the presence of lymphoblasts that has led some to the conclusion that *all* lymphoid cells are primitive cells; while the presence of mature lymphocytes led others to believe *all* blood lymphoid cells to be true lymphocytes, that is, the end cells of the lymphoid series.

SUMMARY

In contrast to the general belief that the lymphoid cells of the peripheral blood of normal animals are end cells, the present study shows that irrespective of their size they are actually composed of lymphoblasts, immature lymphocytes and lymphocytes. In the blood of rats infected with *Trypanosoma brucei* the number of lymphoblasts shows a marked increase on the fifth day of infection, as a result of proliferation of the normally existing, circulating lymphoblasts, and emigration of tissue lymphoblasts from the spleen and lymph nodes. After the 6th day this number decreases, in spite of the increased severity of the disease, probably as a result of increased cell destruction or exhaustion of their proliferative power.

A new classification of the non-granular white blood cells of the rat and a text-figure illustrating the relation between the tissue and blood lymphoid cells are presented in the text.

* Such a separation of the blood lymphoid cells is also possible in normal rabbits and human beings.

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DESCRIPTION OF PLATE

PLATE 42

All the cells illustrated in the colored plate were drawn within 10 minutes after the films were made. They were from the blood obtained by puncturing the saphenous veins of 6 normal and 8 infected rats. Cells from infected animals have their numbers printed in arabic type, those of the normal animals in italics. The size of a red blood cell of the rat is given for comparison.

The following types of cells are illustrated:

Small lymphoblasts — 1-9.

Small immature lymphocytes — 10-19.

Atypical small immature lymphocyte — 20.

Small lymphocytes — 21-30.

(Cell No. 24 underwent degeneration and later appeared as cell No. 25.)

Atypical small lymphocytes — 31-37.

Medium sized lymphoblasts — 38-41.

Medium sized immature lymphocytes — 42-44.

Atypical medium sized immature lymphocytes — 45-48.

Medium sized lymphocytes — 49-54.

Atypical medium sized lymphocytes — 55, 56.

Large lymphoblasts — 57-64.

Large lymphoblast in mitosis — 65.

Large plasma cell — 66.

Large immature lymphocytes — 67-70.

Atypical large immature lymphocytes — 71-75.

Large lymphocytes — 76, 77.

Atypical large lymphocytes — 78-86.

(Cell No. 79 was in active motion; in a few minutes it appeared as cell No. 80.)

Monocyte-like cells — 87-90.

THE HEART VALVES AND MUSCLE IN EXPERIMENTAL SCURVY WITH SUPERIMPOSED INFECTION *

WITH NOTES ON THE SIMILARITY OF THE LESIONS TO THOSE OF RHEUMATIC FEVER

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In experiments designed primarily to study mesenchymal reactions in scorbutic guinea pigs subjected to infection certain degenerative and proliferative lesions were observed in the heart valves. These observations led to a series of experiments directed to the determination of the effect upon the heart valves and muscle of the following: (1) uncomplicated scurvy, acute and chronic; (2) scurvy combined with infection, and (3) infection alone.

METHOD AND PROCEDURE

Diet: The diet used was a slight modification of one used by Dalldorf.¹ Essentially similar diets have been employed by a number of investigators. The exact composition of the diet used in the experiments is set forth below.

The Basal Diet Employed in Experiments

	Per cent
Baked skimmed milk powder (baked at 110 to 120°C for 2 hours)	30.0
Ground rolled oats and bran — equal parts by volume	56.0
Butter fat	10.0
Dried yeast (Fleischmann's yeast for animals and poultry)	1.5
Cod liver oil (standardized)	1.0
Sodium chloride	1.0
Ferrous lactate	0.5

All ingredients were intimately mixed. The mixed food was kept in the refrigerator. This basal diet and water were given *ad lib*.

The diet lacks only vitamin C. It has proved quite adequate for growth and maintenance when supplemented with orange juice. One control animal has

* Supported in part by the Christine Breon Fund for Medical Research.

Read before the American Association of Pathologists and Bacteriologists, May 9, 1933, at Washington, D. C.

Received for publication June 26, 1933.

been so maintained in excellent condition for 6 months. Orange juice was given as desired in measured amounts by pipette.

The Infecting Agent and Method of Infection

The infecting organism used in the bulk of the experiments here reported was a *beta* hemolytic streptococcus, which is the causative agent of spontaneously occurring cervical lymphadenitis frequently encountered in guinea pigs. The organism is a favorable one in that it is readily available, the infection is easily transmitted and the strains used produce a chronic localized infection that only rarely causes death spontaneously and usually remains confined to regional lymph nodes. Although the streptococci employed were all derived from spontaneous cervical adenitis and were all hemolytic, no attempt was made to use a single strain in the different series of experiments. Infection was transmitted by intracutaneous inoculation of pure cultures of the organism grown on Avery's glucose veal broth. From 0.05 to 1 cc. was inoculated into the skin of the thigh below the groin. A skin pustule develops at the site of inoculation followed shortly by localization, tumefaction, and later suppuration of the regional lymph nodes. Infection in the scorbutic animals in general gave larger lesions which persisted longer and were less well encapsulated and localized.

Care was exercised to secure animals without evidence of spontaneous infection. Infected and non-infected animals were kept in separate rooms.

Pathological Examination

Complete postmortem examinations were performed in the majority of animals. Particular attention was given to the heart examinations and many sections were taken from each heart. In most instances the hearts were incised sufficiently to permit penetration of the fixative. (Zenker's solution was employed in most cases.) The hearts were usually trimmed, after being placed in 80 per cent alcohol, in such a way that the mitral and tricuspid valves would be sectioned simultaneously, and later the aortic and pulmonary valves. It was not possible to do serial sections on the hearts but large numbers of sections were cut. The mitral valve received the greatest attention. In several instances entirely inadequate sections were obtained, due to faults in trimming, embedding or sectioning. In spite of this, sections obtained are considered quite adequate for interpretation. Somewhat more than 5000 sections of hearts were examined.

THE EXPERIMENTS

Four series of experiments were conducted — each series comprising groups of 18 to 30 animals. A broad outline of the groups in each series of experiments is given below, tabulating in brief the material upon which pathological studies were made. Representative protocols of each group, giving detail of procedure, are appended at the end of the paper.

Symbols

Control = basal diet supplemented with adequate amounts of orange juice.

Infection only = animals infected and maintained on basal diet supplemented with adequate amounts of orange juice.

Total scurvy = basal diet without supplement.

Subacute scurvy = basal diet supplemented with inadequate amounts of orange juice.

Chronic scurvy = basal diet supplemented with inadequate amounts of orange juice. Animals maintained longer than subacute group. In the present series of experiments the vitamin C deficiencies were rather severe.

TABLE I
Experiment I

No. of animals	Status	Series No.
2	Control	65-66
4	Total scurvy	47-48-49-50
5	Total scurvy plus infection (<i>beta streptococcus</i>) ...	53-54-55-57-58
3	Infection only (<i>beta streptococcus</i>)	59-61-62
1	Infection plus scurvy — late (<i>beta streptococcus</i>) ..	60
1	Infection only — subcutaneous (<i>B. aertrycke</i>)	63
1	Infection with terminal scurvy (<i>B. aertrycke</i>)	64
2	Total scurvy plus infection — subcutaneous (<i>B. aertrycke</i>)	51-52
2	Infection only — intravenous (<i>B. aertrycke</i>)	69-70
2	Subacute scurvy plus infection — intravenous (<i>B. aertrycke</i>)	67-68

TABLE II
Experiment II

No. of animals	Status	Series No.
4	Control	75-78-79-80
6	Infection only (<i>beta streptococcus</i>)	71-72-73-74-76-77
3	Total scurvy	86-87-90
6	Total scurvy plus infection (<i>beta streptococcus</i>) ...	81-83-84-85-88-89

Note: Animals in this series were infected 8 days after being placed on the basal diet. The animals of this group were small, ranging from 185 to 270 gm. Lesions were less well defined in this group because of the short duration of life of very young pigs with scurvy. Animals averaging about 350 gm. are more satisfactory. Animals 71 and 84 received nucleotide K-96 to study blood response.

TABLE III
Experiment III

No. of animals	Status	Series No.
2	Control	104-106
4	Infection only (<i>beta</i> streptococcus)	101-102-103-104-105
2	Subacute scurvy only	109-110
3	Subacute scurvy plus infection (<i>beta</i> streptococcus)	107-108-111
1	Chronic scurvy — terminal total scurvy	114
1	Chronic scurvy only	117
2	Chronic scurvy plus infection (<i>beta</i> streptococcus)	115-118
2	Chronic scurvy plus infection — terminal total scurvy (<i>beta</i> streptococcus)	119-120

Note: In this experiment the infected animals were inoculated at the same time with 0.05 cc. of a 28 hour broth culture. This organism produced minimal infections and in some instances none. The entire group was reinoculated 6 days later in the opposite thigh with 0.1 cc. of another hemolytic streptococcus from a different source.

TABLE IV
Experiment IV

No. of animals	Status	Series No.
2	Control	130-131
4	Chronic scurvy only	146-147-148-149
7	Infection only (<i>beta</i> streptococcus)	132-133-134-135-136-137-138
4	Chronic scurvy plus infection (<i>beta</i> streptococcus) adequate survival	150-151-152-156
3	Chronic scurvy plus infection (<i>beta</i> streptococcus) short survival	153-154-155
2	Infection only (<i>beta</i> streptococcus) reinoculation ...	139-140
2	Chronic scurvy plus infection (<i>beta</i> streptococcus) reinoculation	157-159
4	Infection (<i>beta</i> streptococcus) followed by subacute scurvy	142-143-144-145

Note: More detailed data on precise treatment of individual animals may be had by referring to appended protocols. The chronic scurvy animals subjected to superimposed infection that survived infection for longer periods showed in general more definite pathological pictures. We feel that there is no significant difference in the reinoculated groups. The local reactions to reinoculation were mild, of short duration and showed no evidence of spread.

For the sake of clarity the findings in the four series will be considered as a whole. The results in each series of animals are consistent enough in principle to

warrant this method of consideration. In the heart examination particular attention was directed to degenerative changes in the fiber substance of the valves, to proliferative reactions on the part of the stroma or endothelial cells, and to degenerative and proliferative lesions in the muscle and pericardium. Findings in the heart valves will be considered first.

THE NORMAL HEART VALVES

The normal heart valves present a characteristic structure. Eight control animals and, because in most instances they were normal, 17 animals subjected to uncomplicated infection, comprise the material for this analysis. Sections of the heart were obtained in all. Examination may be considered adequate in about 20 instances.

Control Animals: The normal valves as seen in the control animals possess a compact rich fiber structure. The fibrous stroma is closely set and consists of abundant, wavy, uninterrupted fibrils. The nuclei are of a mature sort, are regular in size and contour and are usually arranged axially. Little or no cytoplasm is visible around the nuclei, which appear to be naked in the fibrous stroma. Of the 8 control animals maintained on the basal diet supplemented with adequate amounts of orange juice none showed significant degenerative or proliferative reactions. Sections of each of these hearts were available for study and may be considered adequate in 5. In 1 animal a very mild proliferation of the endocardial layer was seen overlying the insertion of one of the large chordae tendineae.

Infection Only: The animals on the basal diet supplemented with orange juice and experimentally infected with *beta* streptococcus presented essentially normal valves, except as noted below. One animal showed an acute necrotizing mitral valvulitis, the second an accumulation of a few polymorphonuclear leukocytes near the base of the tricuspid valve with some associated capillary endothelial hyperplasia. Another animal declined with uremia from an obstructed urinary tract. In this animal there was a peculiar degeneration of the collagen fibers of a sort different from that to be described. In a few other instances there was observed a scattering of free mononuclear cells (one with a very mild interstitial proliferation) in the mitral valve and questionable foci of edema or degeneration. It could not be determined with certainty if these were true areas of edema, degeneration or artefacts. No associated proliferative reactions were seen, except the very mild reaction noted above. Of 20

animals in this group sections of the heart were secured in all and sections could be considered reasonably adequate in 15 instances. No changes were seen comparable in type or extent to those that occurred in the groups described below. Figure 1 illustrates an essentially normal aortic valve.

THE HEART VALVES IN SCURVY

In practically all of the animals subjected to acute, severe sub-acute, or chronic scurvy definite degenerative changes in the structure of the valves are noted. The normally rich fibrous structure becomes impoverished. The fibers show thinning, fragmentation and disorganization of their regular axial arrangement. A loss of the normal wavy contours and at times a hyaline degenerative change of the fiber substance is observed. Eight of 9 hearts adequately examined in uncomplicated scurvy reveal changes of this sort.* Another animal in which but few sections are available also shows degenerative lesions. Nuclei of the stroma cells frequently present pyknotic shrunken contours. In addition to the collagen atrophy and degeneration described, in at least 2 instances a mild but definitely proliferative reaction of the endothelial or subendothelial cells is observed in uncomplicated scurvy.

Figure 2 illustrates the typical degenerative changes described, together with a mild proliferative reaction on the part of the endothelial and subendothelial cells. This is the most definite proliferative reaction that is seen in uncomplicated scurvy. Usually only the degenerative changes are present.

THE HEART VALVES IN COMBINED SCURVY AND INFECTION

The lesions produced in the heart valves in combined scurvy and infection are striking; they are characterized by degenerative changes with large cell proliferative reactions on the part of the stroma cells. Frequently masses or fragments of peculiar hyaline material are intermingled with the reacting cells. Fundamentally the lesions produced are similar, though differing individually in precise histology.

* This is in agreement with the fundamental observations of Hojer² who, although he did not describe the heart valves, observed atrophic connective tissue with poorly developed or imperfect collagen in various organs of animals subjected to scurvy. He observed these changes in early and relatively mild degrees of scurvy.

Scurvy and infection were combined in various ways as indicated in the experimental outlines and appended protocols. Of 31 animals subjected to one or another combination of scurvy and infection with *beta* streptococcus reasonably adequate examination of the heart was secured in 24 instances. Of this group all show recognizable degenerative changes in one or more heart valves.* In addition to the degenerative changes 16 hearts show moderate or marked proliferative reactions on the part of the stroma cells in one or more valves. Gross examination of the guinea pig's heart is difficult and unsatisfactory. However, the valves were examined in a few instances. Figure 3 shows a rim of elevated lesions along the line of closure of the mitral valve in an animal subjected to infection followed by scurvy. Figure 4 illustrates a leaflet of an aortic valve showing a diffuse swelling of the valve with a mucoid degenerative appearance of the stroma and a distinct nodular proliferative reaction at the line of closure. In the region of the greatest proliferative activity fragments of an eosinophilic hyaline material are visible. Figure 5 shows an essentially similar reaction in the mitral valve of another animal. Here again hyaline fragments are seen about the proliferating cells and a small, newly formed capillary is present in the midportion of the valve. In one instance, Figure 6, the proliferative reaction is unusually intense, overshadowing any degenerative changes. In another portion of this valve, however, shown in Figure 7, a definite hyaline degeneration is apparent in conjunction with a large cell proliferative reaction. The character of the proliferating cells is shown well, and it may be seen that some of them are multinucleated. Figure 8 illustrates a lesion in which a large amount of hyaline material lying within the substance of the mitral valve, together with the associated proliferation, forms a verrucous lesion at the line of closure. Figure 9, a higher magnification of the same valve, shows the dark, homogeneous eosinophilic hyaline material, the paler mucoid material and the proliferating cells, which make up the lesion. In Figure 10 is shown the mitral valve in an animal subjected to partial scurvy and infection. An area of hyaline degeneration with an accompanying proliferative reaction is seen in one leaflet. Here the endothelial surface layer has been eroded. There is a small focus of disintegrating red cells just beneath the surface. The

* In this study the mitral valve was most closely examined and in most though not all instances showed the maximal involvement.

distal portion of the valve shows atrophic degenerative changes in the collagen. The opposite leaflet presents hyaline degeneration with a mild diffuse hypertrophy and hyperplasia of the stroma cells. A striking lesion of the aortic valve is seen in another animal of the series, Figure 11. Here we find the normal fibrous structure is gone and replaced by cells with a large cytoplasm, some of which show two or more nuclei. At the contact surface is a thin fibrinous mesh. The two leaflets appear almost fused. Numerous fragments of an unidentified hyaline material resembling swollen collagen may be seen in the substance of the valve beneath the contact surface. The cytological characters of the proliferated cells are shown in Figure 12, taken at the contact area of the same valve. The proliferated cells in the various lesions present certain characteristic appearances: They show one or more large hyperchromatic or vesicular nuclei with sharp nuclear membranes and frequently prominent nucleoli or chromatin dots and ridges. The cytoplasm takes a dull eosinophilic or basophilic stain, is usually considerable in amount and outlined poorly, yielding cells of various shapes and irregular contours.

SIMILARITY OF THE EXPERIMENTAL LESIONS TO THOSE OF RHEUMATIC ENDOCARDITIS

Attention is called to the similarity of the experimental lesions described to those of the early stages of rheumatic fever. Swift ³ has directed attention to the interstitial position of the fundamental lesion in rheumatic endocarditis. Clawson ⁴ has emphasized the essential proliferative nature of the inflammatory process in the valves in rheumatic endocarditis. In Poynton and Schlesinger's recent review of rheumatism ⁵ they state: "Evidence is rapidly accumulating in favor of Poynton and Paine's view that the valvular vegetations owe their formation to a subendothelial proliferation of cells and not to a primary destruction of the endothelium." Ribbert ⁶ speaks of characteristic changes in the subendocardial tissues beneath the thrombus or beneath the free surfaces, consisting in an enlargement of and gradual increase in number of the cells, whose cytoplasm enlarges and consequently causes a mild thickening of the tissues. This change, he finds, is accompanied by a transformation of the *zwischen-substanz* which becomes clearer, more translu-

cent and the fibrillar structure less distinct. The essential similarity of the experimental lesions to the early lesions of rheumatic fever is apparent. Perhaps the most exhaustive study of the pathology of rheumatic fever has recently been made by Klinge and his collaborators in a series of twelve papers on "Das Gewebsbild des fieberhaften Rheumatismus." The findings have been summarized in the last publication.⁷ The fundamental lesion they describe as "a swelling of the ground substance of the connective tissue with a simultaneous chemical transformation into a substance which for the most part takes the stain of fibrin." This change, which they briefly designate as fibrinoid degeneration, is considered the primary lesion of rheumatic fever. In the heart valves, as in other connective tissues, there follows a proliferation of the connective tissue cells which, with a gradual disappearance of the fibrinoid masses, dominates the picture so that a part or the whole of the valve is transformed into a cell-rich granulation tissue. This comes close to the pathology observed in the experimental animals subjected to scurvy and infection. Klinge describes as classically rheumatic the verrucous lesion formed by an edematous, mucoid, hyaline, fibrinoid swelling of the valvular tissue (particularly the subendothelial layer), which pushes the endothelium before it forming a warty nodule. The above appears to describe perfectly the experimental lesion illustrated in Figures 8 and 9. This lesion is strikingly like a verrucous nodule shown in Figure 5 of Clawson's⁴ study of endocarditis. Attention is called to the nuclear and cytoplasmic characteristics of the proliferating cells, as shown in Figures 7 and 12, which answer very closely the descriptions given by Gross, Loewe and Eliasoph⁸ of the typical cells in the Aschoff reaction.

We have been unable to date to examine lesions extensively for the presence of bacteria. However, the Giemsa stain, on Zenker-fixed tissue, was applied in one of the most intense proliferative reactions and microorganisms could not be demonstrated.

Briefly, in summary, it may be said that animals subjected to combined scurvy and infection with *beta* streptococcus develop, in a considerable proportion, lesions in the heart valves resembling closely the lesions of rheumatic endocarditis.

Interstitial Reactions: The Aschoff reaction in its most characteristic form is perhaps pathognomonic of rheumatic fever. It is well to remember however, as has been noted by Gross and Ehrlich,⁹ Swift,¹⁰

Clawson,¹¹ Klinge,⁷ and others, that the Aschoff body is not a static structure but goes through an evolutionary cycle and that a considerable factor of time is probably necessary for the development of the lesion in its most characteristic form. In principle and character the lesions produced in the heart valves are fundamentally similar to those of the Aschoff reaction. Judged by the most severe standards it perhaps cannot be fairly said that the Aschoff body has been reproduced in the heart muscle. However, proliferative lesions have been observed in the myocardium and beneath the mural endocardium in animals subjected to a combination of scurvy and infection, which bear a strong resemblance to and are believed to be fundamentally similar to reactions seen in rheumatic fever. These reactions are clearly different from the accumulations of lymphocytes with or without large mononuclear cells and foci of more or less mature fibroblasts occasionally encountered in control animals, or in animals with infection maintained on an adequate diet. Characteristic proliferative lesions produced experimentally in combined scurvy and infection are illustrated in Figures 13, 14 and 15. Degenerative changes are usually discernible in the near-by muscle fibers. Figure 13 shows a well defined proliferative lesion at the angle of attachment of the mitral valve, a frequent site of lesions in rheumatic fever. Figure 14 shows a proliferative focus beneath the mural endocardium. In one area a focus of eosinophilic hyaline material resembling swollen fragmented collagen is seen, and in another area there is a scattering of red blood cells. Figure 15 shows a large cell proliferative response in the muscle of an animal infected and then maintained 38 days on a low vitamin C diet. The fibrinoid degeneration of Klinge has been encountered in the experimental animals in several situations; notably in the synovial and capsular tissues of joints, about the costochondral junctures, in a subcutaneous nodule in one animal and in the interstitial tissues of the heart in another. The latter lesion is shown in Figure 16. Finally, well defined proliferative reactions of large, and at times multinucleated, cells have been observed in the pericardium in several instances, occurring both in uncomplicated chronic scurvy and in scurvy with superimposed infection.

Experiments with B. Aertrycke: The experimental studies using organisms other than the *beta* streptococcus are not sufficient for final conclusions but a few observations with *B. aertrycke* as the

infecting agent suggest that the factor of infection in production of lesions is not specific. Two animals were maintained on the basal diet and subjected to infection by subcutaneous inoculation with *B. aertrycke* 18 days after onset. One animal surviving the infection only 6 days showed some atrophy and collagen degeneration and a small focus of proliferation in the mitral valve. The second animal survived infection 20 days and showed collagen degeneration, a distinct proliferative reaction at the base of the mitral valve, and an Aschoff-like reaction in the muscle at the region of attachment of the mitral valve. An animal, given an adequately supplemented diet and subject to the same type of infection for a similar period, showed essentially normal valves. Two animals were subjected to severe but not total scurvy and infection by intravenous inoculation with *B. aertrycke*. One developed an acute necrotizing and hemorrhagic mitral valvulitis, the other showed definite collagen degeneration and a diffuse interstitial proliferative reaction in the mitral valve. Unfortunately no sections were secured in the 2 control animals of this series. The data for this organism remain incomplete and inadequate but suggest that the infection need not necessarily be specific.

DISCUSSION

Experimental Attempts to Reproduce Rheumatic Fever: Experimental attempts to reproduce the disease rheumatic fever have proved disappointing. No attempt will be made to review the rather extensive literature since the subject has been summarized recently by Gross, Loewe and Eliasoph,⁸ who themselves conducted rather elaborate experiments in an attempt to reproduce rheumatic fever. They conclude: "Judged by the criteria used, we have failed to reproduce the disease. This conclusion we believe holds true for all the work thus far reported in the literature." Studies for the most part have been made with a variety of streptococci isolated in various ways from cases of acute rheumatic fever. While it is true that endocarditis and arthritis have been produced by injecting large doses of streptococci intravenously, such procedures obviously differ rather radically from clinical conditions of rheumatic fever. Although organisms may be found at times in the blood stream in acute rheumatic fever there is no evidence of overwhelming bacteremia. The lesions produced experimentally by such massive intravenous

inoculations with streptococci have corresponded more closely to vegetative endocarditis and suppurative arthritis than to those of rheumatic fever. It is believed that the lesions produced in the heart valves, muscle and pericardium by the experimental method employed in this study correspond more closely to those of rheumatic fever than any previously reported.

The Similarity of the Fundamental Pathology of Scurvy and Rheumatic Fever: Numerous investigations, notably those of Aschoff and Koch,¹² Hojer,² and Wolbach and Howe,¹³ have revealed the basic defect of the scorbutic animal to be an inability to form normal intercellular substances, such as bone, dentine, collagen, and so on. Degenerative changes overtake the preformed collagen and the organism is unable to replace it with a normal substance. The reparative attempt is abortive and an imperfect cement substance is formed. It is of great interest to note that the concept is developing that the fundamental lesion of rheumatic fever is an abnormality of, or degenerative change involving, the intercellular substance of the connective tissue. This has been stressed by Klinge and described as a fibrinoid swelling of the ground substance. It has been previously noted that such lesions corresponding closely to Klinge's descriptions and illustrations were observed in various sites in the experimental animals. Shaw¹⁴ and others have pointed out that masses or fragments of swollen collagen are practically always demonstrable at the center of the rheumatic proliferative reactions. It is quite clear that the dominant lesions of rheumatic fever, *i. e.*, endocarditis, arthritis and subcutaneous nodules, occur at points of stress. It would be at such sites that any weakness or imperfection in the intercellular substance of the connective tissue would first become manifest. It would appear from the experimental studies that the infection alone or the scorbutic state alone are in themselves not adequate for development of the characteristic lesions of rheumatic fever, but that when the insult of infection is added to the fundamental disability of the scorbutic state the lesions occur.

The Factor of Infection in Rheumatic Fever: The vast amount of clinical and bacteriological data that have accumulated would appear certainly to indicate a factor of infection in rheumatic fever and almost certain to implicate one or another form of streptococci. The bacteriological observations on rheumatic fever are extensive. The essential contributions to the bacteriology of this disease are avail-

able in recent reviews by Coburn,¹⁵ Swift,¹⁰ and Poynton and Schlesinger.⁵ All studies point strongly to streptococci, but there is no uniformity in the type or strains described. To explain rheumatic fever simply on the basis of streptococcic infection is inadequate in several respects, notably the unique pathology of the disease, the failure to reproduce the disease experimentally with simple streptococcic infection and finally the lack of uniformity in the type and strain of organisms found associated with it. With a concept of a condition of vitamin C undernutrition as the essential background for rheumatic fever the need for a strain specific infection would appear unnecessary.

Recent investigations of Coburn,¹⁵ Swift,¹⁰ and Gibson and Thomson¹⁶ point rather strongly to the practical importance of *Streptococcus hemolyticus*. Coburn¹⁵ recently studied and reviewed the factor of infection in the rheumatic state. He has observed the prevalence of *Streptococcus hemolyticus* infection in New York in the spring months and makes the following significant statement. "When there was actual local disease resulting from the infection with *Streptococcus hemolyticus*, the clinical picture was manifest as scarlet fever, pharyngitis, tonsillitis, sinusitis, otitis, mastoiditis, cervical adenitis or bronchopneumonia, depending upon the tissue involved and the nature of the clinical response. In most individuals the local disease was the only manifestation of invasion of the tissues with *Streptococcus hemolyticus*. In a small minority, however, local infection was followed by the rheumatic state." This, together with observations by many others, suggests an undefined state occurring in certain individuals which predisposes them to rheumatic fever. Gibson and Thomson find an association between infection with *Streptococcus hemolyticus* and rheumatic fever, but state: "There is some other factor as yet elusive which is required to complete the picture." The observations here reported, together with other preliminary experimental observations on the joints and a study of the epidemiological peculiarities of rheumatic fever,¹⁷ notably the social and seasonal incidence and geographic distribution, strongly suggest that a nutritional state of latent or submanifest scurvy may be the factor predisposing certain individuals to develop rheumatic fever under the added stress of infection. That vitamin C malnutrition exists as a problem of definite importance has been indicated by the work of Göthlin¹⁸ in Sweden, and Dalldorf¹⁹ in this country.

REPRESENTATIVE PROTOCOLS

SERIES I

Control: No. 66. Put on basal diet 8/1/32. Weight 300 gm. Given orange juice supplement 4 cc. daily by pipette. Excellent condition when chloroformed on 8/29/32. Weight 375 gm.

Pathological notes: Tissues normal.

Infection Only: No. 61. Put on basal diet 8/1/32. Weight 375 gm. Given daily adequate orange juice supplement. 8/16/32, infected with *beta* streptococcus 0.1 cc. intracutaneously in thigh (inner aspect). 8/19/32, small pustule and regional enlarged lymph node. 8/30/32, skin healed, 2 small, regional lymph nodes. 9/6/32, chloroformed.

Pathological notes: Encapsulated suppurated inguinal lymph nodes. Other tissues essentially normal.

Total Scurvy: No. 49. Put on basal diet 8/1/32. Weight 300 gm. Maintained without orange juice supplement until death 9/2/32. Weight 190 gm.

Pathological notes: No evidence of infection. Collagen degeneration and slight proliferation in mitral valve (Fig. 2). Hemorrhage into bladder. Erythrophagocytosis in lymph nodes.

Total Scurvy Plus Infection: (*beta* streptococcus) No. 53. Placed on basal diet only 8/1/32, weight 340 gm. 8/16/32, weight 390 gm. Inoculated intracutaneously skin of thigh with 0.1 cc. 24 hour culture *beta* streptococcus. 8/19/32, pustule with small regional lymph node. 8/26/32, large local mass of lymph nodes with reddened, fixed overlying skin. 9/5/32, animal given 2 cc. orange juice. 9/6/32, sacrificed. Weight 210 gm.

Pathological notes: Necrotic area in left groin surrounded by enlarged infected lymph nodes fixed to surrounding tissues. Mitral valve shows typical verrucous lesion (Fig. 8). Spleen enlarged, hyperplastic pulp, microscopic abscesses, early fibrosis of malpighian bodies. Lungs, marked passive congestion and edema. Hemorrhage into adrenals and bladder. Costochondral junctures show typical scorbutic changes, one microscopic focus of osteomyelitis. Fibrinoid degeneration in heart and about costochondral junctures.

Subacute Scurvy Plus Infection: (*B. Aertrycke* intravenously) No. 67. Placed on basal diet 8/18/32. So maintained without orange juice for 21 days. Then partial scurvy for the next 21 days to 9/28/32 (total 14 cc. orange juice in divided doses). 9/1/32, inoculated into the skin with *alpha* streptococcus (human source). Did not develop any infection. 9/14/32, 0.2 cc. of broth culture of *B. aertrycke* given intravenously. Died 14 days later on 9/28/32.

Pathological notes: Abscess in liver, fibrinous pleurisy with collapse of left lung. Hemorrhage into adrenals and swollen costochondral junctures. Microscopic examination shows a degenerative and proliferative lesion in the mitral valve and a slight pericardial proliferative reaction; purulent empyema with subpleural abscesses; focal necrosis in the liver; fibrosis of malpighian bodies in the spleen.

SERIES II

This series was essentially similar to Series I. The infecting agent was a *beta* streptococcus. The group of animals was younger and the pathological changes less definite, due to the shorter survival period of younger animals.

SERIES III

Infection Only: (beta streptococcus) No. 105. Placed on basal diet 12/1/32. Weight 330 gm. Received daily orange juice supplement throughout experiment. 12/15/32, inoculated intracutaneously (left thigh) with 0.1 cc. broth culture *beta streptococcus*. 12/21/32, showed small nodule at site of inoculation. No involvement of regional node. Reinoculated right thigh with 0.1 cc. broth culture *beta streptococcus* from another infected animal. Developed skin reaction and regional lymph node infection, which reached maximum size of 1.5 cm. by 1 cm. on 12/27/32. Also a smaller node in the right groin. Subsequent course: regression of lesions. Chloroformed 1/31/33.

Pathological notes: Encapsulated lymph node 0.5 cm. in diameter in left groin. Other gross findings normal. Questionable edema in leaflet of mitral valve. Focal accumulation of lymphocytes in heart muscle.

Subacute Scurvy Plus Infection: (beta streptococcus) No. 111. Placed on basal diet without orange juice 12/1/32. Weight 315 gm. 12/15/32, inoculated intracutaneously 0.05 cc. 28 hour broth culture *beta streptococcus*, left thigh (inner aspect). 12/21/32, small lymph node enlargement. Reinfected with 0.1 cc. broth culture of *beta streptococcus* of different source by intracutaneous inoculation in right thigh. Developed enlarged regional lymph node, which subsided after maximum enlargement on 12/27/32. Both knees swollen 12/24/32. Beginning 12/19/32 over period of 11 days was given total of 18 cc. orange juice in divided doses. 1 cc. given 1/4/33 and none further until chloroformed 1/20/33. Weight 254 gm.

Pathological notes: Gross findings — enlarged spleen, moderate hemorrhage about knees and into chest wall on left. Swollen costochondral junctures. Gross examination of mitral valve showed verrucous nodule. Microscopic examination showed degenerative and proliferative lesion of mitral valve (shown in Fig. 10). Spleen shows hyperplastic pulp, erythropoiesis and fibrosis of malphigian bodies.

Chronic Scurvy Plus Infection: (beta streptococcus) No. 118. Weight 365 gm. Placed on basal diet 12/1/32. Duration of experiment 2 months. Received 2 cc. orange juice on alternate days for first month and 1 cc. on alternate days for last month. 12/15/32, inoculated 0.05 cc. culture *beta streptococcus*, intracutaneously left thigh. Developed small skin lesion and small node in groin. 12/21/32, reinoculated right thigh 0.1 cc. *beta streptococcus*. 12/27/32, shows large infected mass in the left groin fixed to surrounding tissue and skin and immobilizing the knee. Smaller lesion on right. Gradual regression of lesions until sacrificed 1/31/33.

Pathological notes: No evidence of active infection. Knees fixed. Nodule noted grossly on aortic valve. Costochondral junctures swollen. Microscopic examination shows striking proliferative lesion on aortic valve (Fig. 11). Fibrinoid degeneration about costochondral junctures. Degenerative changes in intercostal muscle. Fibrosis of malphigian bodies in spleen. Fibrinoid degeneration and proliferation in synovia and periarticular tissue of knee. Little hemorrhage into joint spaces.

SERIES IV

Dietary Control: No. 130. Weight 368 gm. Maintained on basal diet supplement with orange juice (4 to 6 cc. daily) for 71 days when chloroformed. Weight 625 gm. Excellent condition. Joints negative.

Pathological notes: No gross changes. Heart valves normal limits. Slight endothelial proliferation over insertion of one of the large chordae tendineae. Focal accumulation of lymphocytes and nuclear fragments in tissue between auricle and ventricle. Knees histologically normal.

Chronic Scurvy Only: No. 149. Weight 390 gm. Placed on basal diet beginning 1/19/33. Maintained for 71 days with 24 cc. orange juice in divided doses. 2/23/33 showed partial fixation of both knees. Chloroformed 3/31/33. Weight 300 gm.

Pathological notes: Slight hemorrhage into chest wall. Hemorrhagic stippling of bladder. Swollen costochondral junctures. Little hemorrhage into muscle above and below knees. Mitral valve shows moderate degeneration. A small Aschoff-like proliferative reaction is seen in the epicardium. Lymph nodes show erythrophagocytosis and pigmentation. Fibrinoid degeneration about costochondral juncture. Fatty degeneration about central veins of the liver.

Infection Only: (beta streptococcus) No. 132. Weight 411 gm. Placed on basal diet beginning 1/19/33. Maintained for total of 67 days with daily orange juice supplement (4 to 6 cc.). Inoculated 2/10/33 with 0.1 cc. broth culture *beta streptococcus* 22 days after onset. 2/19/33, shows enlarged inguinal lymph node 1.2 by 0.6 cm. Gradual regression of infection until sacrificed 3/27/33. Weight 550 gm.

Pathological notes: Complete regression of local infection. Organs and tissues including joints grossly normal. Microscopic examination: heart valves normal except for questionable area of degeneration in mitral valve. Excess iron pigment in spleen and old degenerative lesion at costochondral juncture (evidence of old scurvy anteceding experiment?). Knee and elbow joints quite normal.

Chronic Scurvy Plus Infection: (beta streptococcus) No. 152. Weight 408 gm. Dietary regimen as in No. 149. Infection as in No. 132. Basal diet for 67 days with total of 24 cc. orange juice in divided doses. Inoculated 0.1 cc. broth culture *beta streptococcus* into skin of left thigh on 2/10/33. 2/19/33, infection maximal with large mass in left groin 2 by 1.3 cm. On this day noted swelling and fixation of right knee and fixation of left knee. Infection persistent, slow regression. Animal chloroformed 3/27/33. Weight 272 gm.

Pathological notes: Small lymph nodes in both groins. Patchy atelectasis of lungs. Costochondral junctures swollen. Single abscess in spleen. Bladder hemorrhagic. Brownish discoloration (old hemorrhage) about right knee. No evidence of recent hemorrhage. Questionable shortening of mitral valve. Subcutaneous nodule over bony prominence of knee. Microscopic examination shows definite interstitial proliferation in mitral valve, shown in Fig. 5. The subcutaneous nodule shows bands of fibrinoid degeneration and endothelial proliferation resembling the lesion of rheumatic fever. Joints show fibrinoid degeneration in synovia and periarticular tissues. Similar degeneration in connective tissues about costochondral junctures. Much hemosiderin in the spleen. Hemosiderin and erythrophagocytosis in cervical lymph nodes.

Infection Followed by Subacute Scurvy: No. 142. Placed on basal diet 1/19/33 and so maintained with adequate orange juice supplement (4 to 5 cc. daily) to 2/10/33, when inoculated with 0.1 cc. culture *beta streptococcus*. Following this, maintained for 40 days with total of 11 cc. orange juice in divided doses. Maximal local infection 2/25/33. Mass in left groin 1.8 by 1 cm.

3/3/33, right knee slightly fixed and swollen. 3/14/33 both knees fixed and swollen. 3/18/33, elbows swollen and tender. 3/22/33, sacrificed. Weight 272 gm.

Pathological notes: Some hemorrhage into chest and abdominal walls and into bladder. Swollen costochondral junctures. Heart: gross lesion mitral valve, Fig. 3; microscopic lesions Figs. 6 and 7. Spleen: pigment in pulp and fibrosis of malphigian bodies. Hyaline material in joint spaces with synovial proliferation. Proliferation of capsular tissues.

SUMMARY

The effect of scurvy, and scurvy combined with infection (*beta* streptococcus), upon the heart valves and muscle in the guinea pig has been studied. Infection in animals maintained on an adequate diet usually produces no significant lesions in the heart valves. When they occur they are of an exudative, rather than proliferative type. In uncomplicated scurvy definite atrophic and degenerative changes occur in the collagenous stroma of the heart valves. In scurvy with added infection striking lesions of a combined degenerative and proliferative character develop in the heart valves with considerable frequency. Attention is directed to the similarity of the experimental endocarditis so produced to that of acute rheumatic fever.

Degenerative and proliferative lesions occur in the heart muscle and pericardium of the experimental animals subjected to combined scurvy and infection which are considered similar in type to the Aschoff reaction.

Brief evidence is given that organisms other than the *beta* streptococcus may, in the presence of scurvy, produce such lesions.

The similarity of the fundamental pathology of scurvy and rheumatic fever is considered. Attention is called to the fact that a basic lesion in both conditions is a degenerative change occurring in collagen. Lesions resembling the "fibrinoid degeneration" of Klinge have been seen in various sites in animals subjected to combined scurvy and infection. Klinge considers this type of degeneration the characteristic and initial lesion of rheumatic fever.

The role of infection in rheumatic fever is briefly discussed. It is pointed out that a factor of infection would appear beyond dispute and that the streptococcus in one form or another is strongly implicated. Much evidence clearly indicates that there is some other

factor than simple infection contributing to the development of rheumatic fever.

Experimentally, infection alone or scurvy alone will not produce significant lesions, but when scurvy and infection are combined striking lesions are produced, particularly in the heart valves.

On the basis of the experimental data reported, and other experimental, epidemiological and clinical data previously outlined, the theory is advanced that a condition of vitamin C undernutrition may be a necessary background for the development of rheumatic fever; when the insult of infection is combined with the scorbutic state, the pathological picture of rheumatic fever develops.

NOTE: The authors wish to express appreciation to Dr. Charles L. Connor for stimulation and advice given during the course of these experiments.

We also wish to acknowledge the valuable technical assistance of Dr. L. L. Ginzton, Mrs. K. L. Purviance, Miss Pearl Y. Hall, Mr. W. A. Hewitt and Mr. E. Nauman. Miss Bernice Eddie generously assisted in the bacteriological work.

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DESCRIPTION OF PLATES

PLATE 43

- FIG. 1. Aortic valve. Control infection. Animal No. 132. Showing compact structure of essentially normal aortic valve. $\times 95$.
- FIG. 2. Mitral valve. Scurvy only. Animal No. 49. Showing degenerative changes in valvular stroma and a mild proliferative reaction. The degenerative changes are characteristic; proliferative reactions are not usual in uncomplicated scurvy. $\times 90$.
- FIG. 3. Mitral valve. Infection plus scurvy. Animal No. 142. Showing rim of elevated nodular lesions along line of closure of the mitral valve. Drawn under dissecting microscope. See Figs. 6 and 7 for microscopic appearances of this valve.
- FIG. 4. Aortic valve. Chronic scurvy plus infection. Animal No. 159. Showing swelling and degeneration of valve with distinct proliferative nodule at line of closure. $\times 100$.

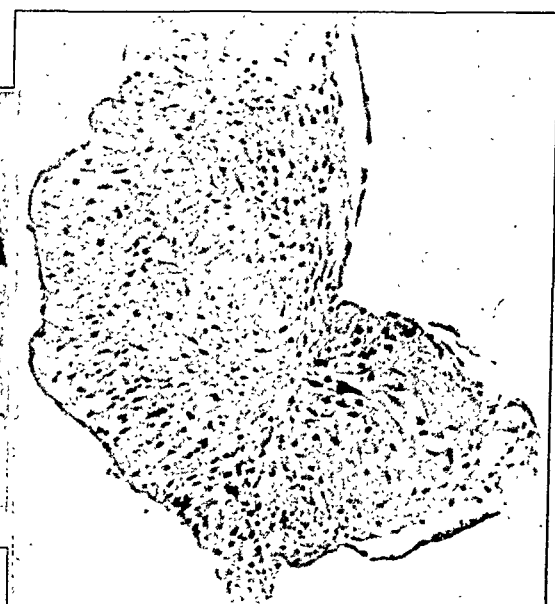


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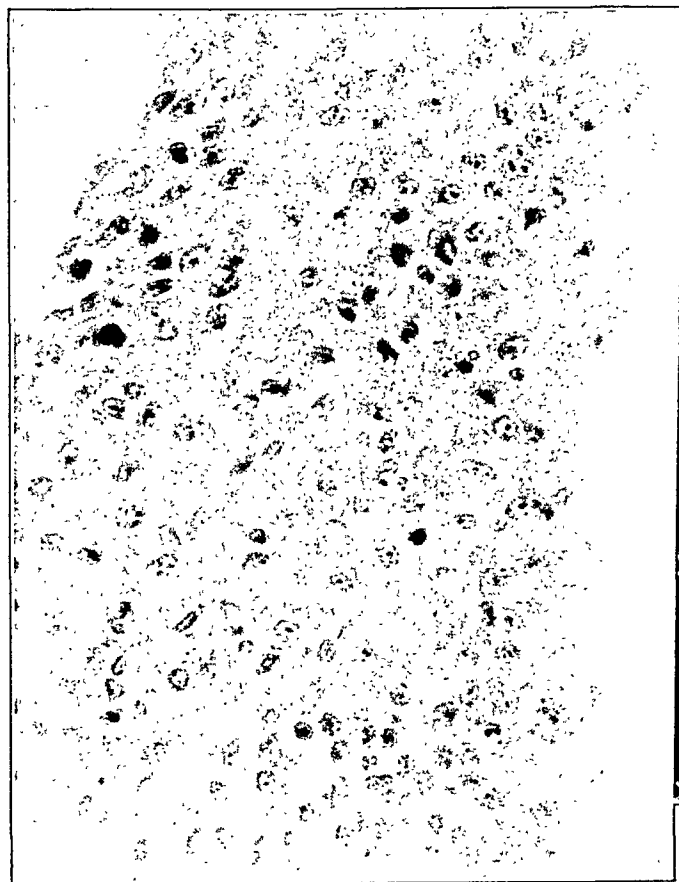
PLATE 44

- FIG. 5. Mitral valve. Chronic scurvy plus infection. Animal No. 152. To show swelling and degeneration in the stroma and a diffuse subendothelial proliferative reaction in the mitral valve. A delicate, newly formed capillary may be seen in the mid portion of the valve. $\times 120$.
- FIG. 6. Mitral valve. Infection plus scurvy. Animal No. 142. An unusually intense proliferative reaction involving the full thickness of the valve. See Fig. 3 for gross appearance. $\times 95$.
- FIG. 7. Mitral valve. Infection plus scurvy. Animal No. 142. Another area of the valve illustrated in Fig. 6. This section shows a hyaline degenerative change in the valve stroma with an accompanying proliferative reaction. Detail of the proliferated cells may be seen. Some are multinucleated. $\times 175$.
- FIG. 8. Mitral valve. Scurvy plus infection. Animal No. 53. A combined degenerative and proliferative lesion in the mitral valve occurring in region of line of closure. See next figure for detail. $\times 45$.



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PLATE 45

FIG. 9. Higher magnification of the lesion illustrated in Fig. 8. A fibrinoid and mucoid degeneration with an early proliferative response composes this verrucous nodule. This lesion is considered characteristically rheumatic. $\times 350$.

FIG. 10. Mitral valve. Subacute scurvy plus infection. Animal No. 111. An area of hyaline degeneration with an accompanying proliferative reaction is seen in one leaflet. Here the endothelial surface layer has been eroded. There is a small focus of disintegrating red blood cells just beneath the surface. The distal portion of this leaflet shows atrophic degenerative changes in the collagen. The opposite leaflet presents hyaline degeneration with a mild diffuse hypertrophy and hyperplasia of the stroma cells. $\times 45$.

FIG. 11. Aortic valve. Chronic scurvy plus infection. Animal No. 118. A striking proliferative reaction in two leaflets of the aortic valve; diffuse edema of valve with proliferation of hyperplastic cells. The two leaflets appear almost fused. Fragments of an unidentified hyaline material resembling swollen collagen are present in the substance of the valve beneath the contact surfaces. A thin layer of fibrin lies between the two leaflets. $\times 95$.

FIG. 12. Higher magnification of same lesion shown in Fig. 11. Showing detailed characteristics of proliferating cells. Note the large vesicular nuclei with prominent chromatin dots and nuclear membranes. The cytoplasm is abundant, dully eosinophilic (hematoxylin and eosin stain), faintly granular and irregularly outlined. Some of the cells show more than one nucleus in a single mass of cytoplasm. $\times 650$.



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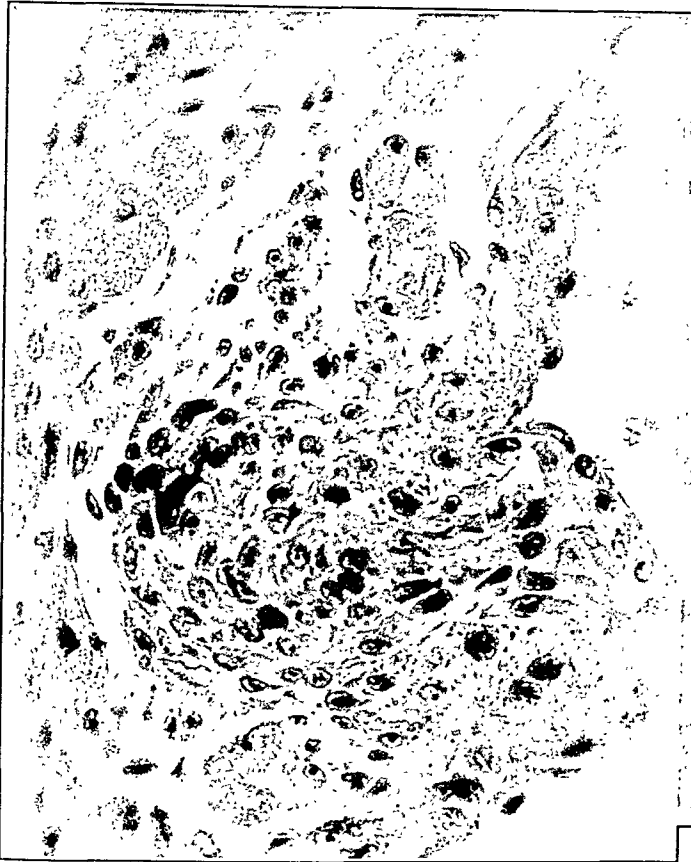


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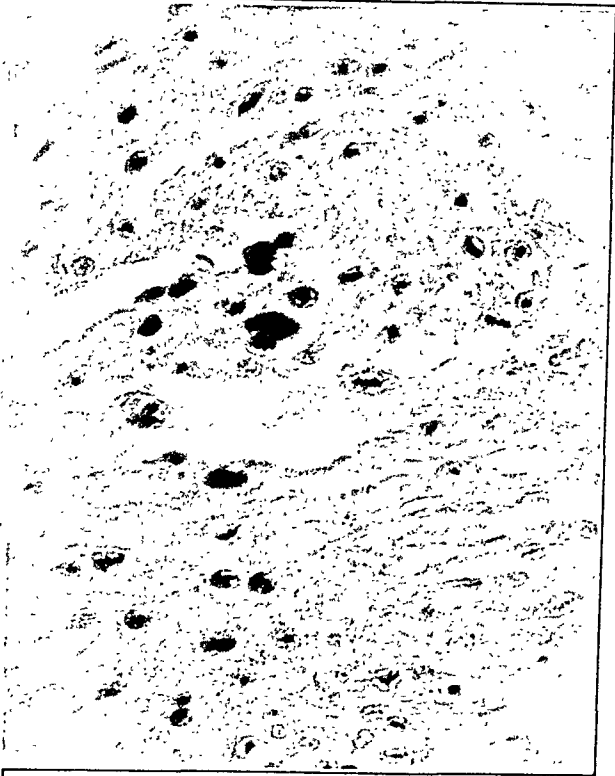
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PLATE 46

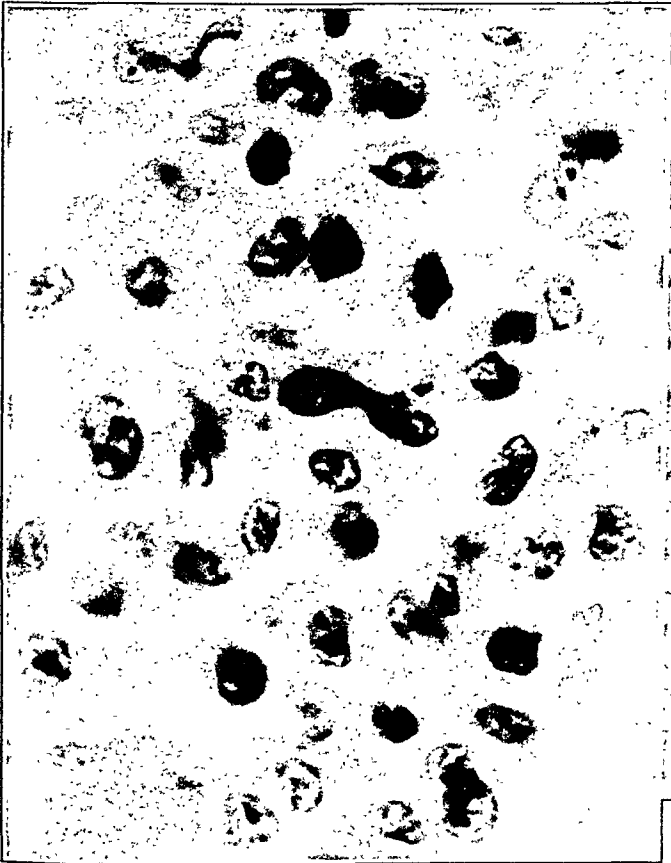
- FIG. 13. Interstitial proliferative reaction at the angle of attachment of the mitral valve. Infection plus scurvy. Animal No. 143. Showing a concentric proliferative nodule composed of hyperplastic cells resembling the Aschoff reaction of rheumatic fever. $\times 420$.
- FIG. 14. Subendocardial reaction. Chronic scurvy plus infection. Animal No. 115. This proliferative reaction beneath the mural endocardium shows in one area a focus of eosinophilic hyaline material resembling swollen collagen, and in another area there is a scattering of red blood cells. The hyperplastic cells resemble those of the Aschoff reaction. $\times 420$.
- FIG. 15. Interstitial proliferative reaction. Infection plus scurvy. Animal No. 145. Showing a portion of an interstitial proliferative reaction. The character of the cells forming the focus is well shown. Some contain more than one nucleus. Although not well shown in the photograph the surrounding muscle fibers show distinct degenerative changes. $\times 560$.
- FIG. 16. Fibrinoid degeneration of collagen in heart. Scurvy plus infection. Animal No. 53. A focus of degenerated collagen, which histologically reacts as fibrin, is shown adjacent to the heart muscle. The change involves the wall of a small vessel in addition to an elongated focus of collagen. A change of this type is considered by Klinge to be the fundamental and earliest lesion of rheumatic fever. $\times 175$.



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REPORT OF A CASE OF NON-LIPOID HISTIOCYTOSIS (RETICULOENDOTHELIOSIS) WITH AUTOPSY *

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INTRODUCTION

During the past decade, following Aschoff's and Kiyono's promulgation of their conception of a reticuloendothelial system, a number of cases have been reported that appear to represent a stimulation of this system, without constituting neoplasia and without showing the lipoid changes of Gaucher's disease or of lipoid histiocytosis. Most of these have appeared in the German literature, coming from German or Russian sources. The titles of the articles vary from "Reticuloendotheliosis" to "Aleukemic Reticulosis," with the use of other terms to express the pathological pictures observed. Theories as to the nature of the disease fall into two categories: the conception that it represents a response to bacterial or toxic stimuli and is, therefore, of an infectious nature, and the idea that it constitutes a third type of leukemia in which the monocyte (or histiocyte) is the type cell. Cases have been reported in which there did appear to be a monocytic leukemia but, on the other hand, many articles have been published in which infection seems most important, and there is no mention of increase of mononuclears in the circulation. We have chosen the term "non-lipoid histiocytosis" as expressing a subdivision in the reticuloendothelioses in general and in contradistinction to lipoid histiocytosis and its nearly related condition, Gaucher's disease, in which lipoids are a prominent feature.

The clinical picture of the disease is very indefinite and poorly characterized. The age and sex of the patients seem to be unimportant, although the picture is somewhat different in childhood from its type in adult life. There is usually a history of recent infection, ranging from grippe or a severe cold, to otitis media, faucial infections

* Read before the New York Pathological Society December 28, 1933.
Received for publication August 11, 1933.

such as Vincent's angina, or infections of the intestinal or genitourinary tracts. The patients show a secondary anemia of varying grade and, in most cases, there is a slight leukocytosis of the polymorphonuclear type, although leukopenias may be noted, as in our case. Fever is usually not a prominent feature, although the temperature is often subfebrile. A history of frequent and sometimes familial epistaxis may be elicited. In children there is almost always a marked and generalized purpuric eruption on the skin with scattered, very small petechiae over the greater part of the body. For this reason thrombocytopenic purpura is often diagnosed, to be subsequently ruled out by further examination.

Physical signs are scanty, except for a variable lymphadenopathy and an almost invariably enlarged spleen. The liver is also usually found to be enlarged in the case of children, while in adults this is not always the case. On roentgenological examination bone changes are frequently seen; it is possible that they are more common than is supposed and could have been demonstrated had they been looked for with the X-ray. Three cases besides ours showed cysts or areas of rarefaction in the bones (Guizetti,¹ Goldzieher and Hornick,² and Schultz, Wermbter and Puhl³); Pentmann's⁴ case showed numerous cavernous hemangiomas, one of which involved a vertebral body.

At autopsy the following pathological changes are usually noted. In children there is a generalized petechial skin eruption; petechial hemorrhages in the epicardium (as distinguished from the pericardium in general) are almost the rule in cases of all ages, and there may also be petechiae in the pleura. The thymus is the site of small abscess-like areas of softening resembling Dubois abscesses and referable to involution and necrosis of the Hassal's corpuscles, as determined by the microscope. The lungs and heart show nothing characteristic. There is usually generalized enlargement of the lymph nodes, the cervical, retroperitoneal and other chains often being involved as groups; sometimes the nodes at the portal region are very large. The liver is practically always enlarged in children and shows extensive fatty infiltration; in adults it may be enlarged, but is not regularly fatty. In both cases early cirrhotic changes are the rule. The spleen is always large, firm and tense, with rounded edges. On section the markings are indistinct and there are sometimes yellowish nodules in the parenchyma. The bone marrow often shows overgrowth into the bony spongiosa, with rarefaction of the

latter that sometimes goes on to cystic degeneration. The changes noted elsewhere in the body are variable and not characteristic.

Microscopically these changes are found to be due to an overgrowth of large mononuclear cells that multiply in the sinuses of the lymphoid apparatus and spleen to an almost neoplastic degree, at the expense of the lymph follicles, and often show marked phagocytic tendencies toward erythrocytes and leukocytes. Sometimes they merely contain hemosiderin or chromatin fragments. These large cells are characterized by a dense, sometimes basophilic cytoplasm, a polyhedral contour, a tendency to lie discretely separated and to form giant cells or syncytia that differ somewhat from those of the Langhans type or the Dorothy Reed (Sternberg) variety. Their nuclei are vesicular and correspond with those of the retothelium (we shall use Roulet's ⁵ abbreviated terminology for reticuloendothelium) in general. They may have one, or several of these. Often there are cells quite indistinguishable from megakaryocytes in both spleen and lymph nodes. None of the type cells is possessed of the clear, longitudinally meshed or striated cytoplasm of the Gaucher cell, or of the foamy, vacuolated body of the Pick-Niemann type. In some cases reported eosinophils and plasma cells have been noted in association with these retothelial cells in moderate or large numbers. The liver shows a similar, but usually much less marked, retothelial proliferation and infiltration in the sinusoids and the portal areas, together with a granulomatous overgrowth of lymphoid tissue in the latter. The Kupffer cells may or may not be involved in the process. The picture here is far less striking than it is in the spleen and lymph nodes. Overgrowth of the connective tissue of the liver is the rule, sometimes amounting to early cirrhosis (there may be central necrosis in adults); in children extreme and generalized fatty infiltration of the parenchyma is the rule. The bone marrow is also the site of similar retothelial overgrowth at the expense of hematopoiesis and there may be considerable increase in the fibrous tissue and vascular content of the marrow with an apparent congestion and hemorrhage. Sometimes there is a gelatinoid degeneration of areas of marrow that invade and destroy the spongiosa and may form cysts.

The origin of the type cells in this disease is generally attributed to the reticuloendothelial apparatus; to enter into a discussion of this would merely reopen a much discussed subject. The participation of the Kupffer cells in the process is usually assumed, but scarcely

proved as yet. We shall discuss the question of etiology later; thus far we have simply attempted to establish criteria for our diagnosis by reference to facts gleaned from the study of some twenty cases reported in the literature. It should be noted, in passing, that only one of these comes from an American source; either the condition is rare in this country, or our pathologists have overlooked it and failed to recognize it.

REPORT OF CASE

Clinical History: The patient was a $2\frac{3}{4}$ year old white female who had been ill for about 1 year, during which she had had red, hemorrhagic spots on the skin. Her father had suffered from hay fever and was subject to frequent nose-bleeds, which was also true of her maternal grandmother; her father's brother had also had frequent epistaxis. The child was a normal, full-term baby. She had had three attacks of catarrhal otitis media and had had frequent colds, together with colitis on one occasion. The spleen had been noted as enlarged 3 months prior to admission and, at that time, she had had severe nasal hemorrhage. Shortly after this she was admitted to the Johns Hopkins Hospital, where a diagnosis of purpura hemorrhagica was made. The blood count showed: red blood cells 3,830,000, hemoglobin 75 per cent, white blood cells 4100, polymorphonuclears 25 per cent, lymphocytes 69 per cent. The smear showed few platelets, the bleeding time was 6 minutes, clotting time $\frac{1}{2}$ minute. Treatment consisted of a transfusion and the family was advised to have a splenectomy performed.

Upon admission to the Department of Pediatrics of the New York Hospital the child's body was found to be covered with tiny punctate petechiae. The liver was palpable 7 cm. below the right costal margin. The spleen was also palpable. The tonsils were large and the inguinal and posterior cervical lymph nodes moderately so. There had been no jaundice. Throat cultures and the Schick and Mantoux tests were negative. The temperature remained below 37.8°C . The first blood count showed: red blood cells 3,700,000, white blood cells 3900, adult polymorphonuclears 30 per cent, immature polymorphonuclears 7 per cent, lymphocytes 39 per cent, mononuclears 4 per cent, and eosinophiles 2 per cent. Two platelet counts showed 80,000 and 130,000 respectively. The clotting and bleeding time were 3 minutes with normal capillary clot. The day after admission it was noted on her chart: "Not a typical case of thrombocytopenic purpura, in view of the marked enlargement of the liver and spleen and the normal bleeding time and clot retraction." Roentgenological examination showed no changes suggestive of Gaucher's disease, but there was some stippling of the lower ends of both humeri.

The child was readmitted about 2 weeks later and a blood examination showed that the platelet count had fallen, while the liver had still further increased in size. Physical examination revealed nothing of note except for the enlarged liver and spleen. The lymph nodes were not much enlarged. Blood counts were done and the platelets found to have fallen to 50,000 to 82,000. The hemoglobin was 80 to 90 per cent. Two transfusions of 150 cc. of blood were administered and about a week after readmission she was transferred to the Department of Surgery, where a small piece of spleen was excised for biopsy. Mi-

microscopic examination of this showed a diminution in the size of the follicles, a profusion of large mononuclear cells in the sinuses (where they were actively phagocytosing erythrocytes) and a slight fibrous increase. A provisional diagnosis of "non-lipoid histiocytosis" was made for the occasion, as the picture was unlike anything we had seen or read about up to that time. The complete microscopic description will follow.

Two weeks later splenectomy was performed in the usual manner and the spleen sent to the laboratory of surgical pathology, where the following description was made.

"The specimen consists of a spleen weighing 310 gm.; it is distended and its capsule is tense, its margins softly rounded and its surface everywhere smooth and shining, with a slight mottling of lighter areas on a dark reddish background. On section it is firm and a good deal of blood escapes. The section-surface shows pearly gray areas about 3 mm. in diameter against a brownish red background.

Microscopically one encounters a very novel and obscure picture (Fig. 1): the capsule and trabeculae are somewhat thicker than normal, there is an increase in the connective tissue in the follicles and radiating from them. Four stains are employed: Masson's hematoxylin-phloxin-saffron, his anilin blue and his light green trichrome, and a combination of Mallory's phosphotungstic acid hematoxylin with a silver impregnation. The size of the Malpighian follicles is decreased, the arterial apparatus seems to be in order and practically all the pathological features are found on the venous side of the circulation.

The venous sinuses and the pulp surrounding them are inundated with large, polyhedral cells with a dense, rather than vacuolated, cytoplasm. This is somewhat basophil in the Masson stains. Their nuclei may be multiple, lobulated and double, or irregular and of the gigantic type; most of them are single and they are always vesicular and correspond with the typical nucleus of the retothelial cell. Many of them contain from one to many phagocytosed erythrocytes and, occasionally, ingested polymorphonuclear leukocytes. The silver impregnation shows these cells to lie in intimate relationship to complexes of reticulin fibrils, which further strengthens the assumption that they are retothelial. There is some increase in the reticular fibers of the splenic framework, particularly in the vicinity of the splenic corpuscles. The large phagocytic cells are not at all similar to those of Gaucher's disease as they are smaller, more discrete, denser and lack the characteristic longitudinal striations of these, as well as

their epithelioid grouping in the sinuses. They also lack the multivacuolated or spongy appearance of the cells seen in lipoid histiocytosis.

Besides the cells just described, one finds a good many eosinophil leukocytes and plasma cells in the pulp and sinuses, which gives the picture a slight cast toward the inflammatory side. Occasionally there is a lining up of the mononuclear cells along the walls of the intermediate venous sinuses and collecting veins, with a consequent similarity to cuboidal epithelium, a phenomenon not infrequently seen in low grade inflammations of the spleen. One more fact remains to be described: the presence of giant forms of mononuclear phagocytes that may be multinuclear, or may possess one very large vesicular nucleus, or a darkly stained and lobulated one, somewhat like that of the Dorothy Reed cell of Hodgkins' granuloma. We therefore have: (a) fibrous increase, (b) eosinophil leukocytes, and (c) cells resembling Dorothy Reed giant cells. Although these three points are characteristic of Hodgkins' disease, one is disinclined to make this diagnosis in the face of the other quite atypical findings and turns, rather, to some sort of inflammatory stimulus for an explanation. In the absence of precedent for diagnosis it would perhaps be best for the present to adhere to the biopsy diagnosis of 'non-lipoid histiocytosis.'"

Postoperative Course: After the splenectomy the platelets rose to 90,000, 120,000 and later to 300,000. The red blood cell count was unchanged, the leukocytes rose to 4000 and 9000 with 55 per cent adult polymorphonuclear and 8 per cent immature. The bleeding and clotting time were, as before, 3 minutes each. A culture taken from the wound before it had closed entirely showed *Staphylococcus aureus hemolyticus*. The child's temperature rarely exceeded 37.6° C, excepting on the 4th day after operation when it was moderately elevated. The blood cholesterol determination was 2.65 gm. per liter. The patient was discharged about 5 weeks after the operation, free from petechiae, and her wound healed over after a suture had come away.

She was readmitted 3 months later to the pediatric service as she had been complaining of pain in the legs after a fall that occurred a week or two earlier; she had also hurt her arm in a fall and it remained painful. She now exhibited a recrudescence of the petechial eruption and the liver had become still larger. A transfusion a week after readmission was followed by an aggravation of the petechial eruption over the face and trunk. Shortly after this a kyphosis was noted, tentatively diagnosed as tuberculous, and she was placed on a Bradford frame. The hip and shoulder joints became tender. There was subfebrile temperature, 37.6° C throughout her second hospitalization until death. A transfusion on June 3rd did not relieve the condition and death occurred on June 8th. She had a pharyngitis on May 16th, shortly after readmission, cultures from which

showed *Staphylococcus aureus hemolyticus* and *Streptococcus hemolyticus*; the staphylococcus was also recovered from the blood. The icteric index and fragility test were normal throughout her stay in the hospital. Roentgenological examination now showed rarefaction of the lower pelvis and of the right femur near its head, with osteoporosis of the spine and reticulation of the vertebral architecture, together with increase in some of the intervertebral spaces.

AUTOPSY REPORT

An autopsy was performed 3 hours postmortem.

Inspection: A well developed and well nourished white female of 3 years, 93 cm. in length and weighing 15 kg. No rigor mortis is present, nor is the kyphosis noted during life apparent. There is no increased flexibility in the vertebral column. Slight edema is present practically universally. The skin is white, except for innumerable small, discrete petechiae on the trunk, especially prominent over the chest. These are very small and distinctly outlined. No cervical adenopathy is noted, although there is slight enlargement of the inguinal nodes. There is no discharge from any of the body openings. A white scar is seen over the left rectus muscle, 12.5 cm. in length.

Incision: The usual Y-shaped median incision is made, as well as one over the length of the right femur; the head is opened in the usual manner. The subcutaneous tissue is edematous throughout and the fatty panniculus scanty. On running the knife parallel to the skin over the anterior thoracic wall a small pocket filled with reddish material of the consistence of cornmeal is encountered. This seems to arise from the right lower edge of the sternum and the adjacent ribs and is 3 cm. in its long and transverse axes and 1.5 cm. in depth. It does not extend to the pleural cavity. Another pocket is found over the lower left ribs; at first, thick chocolate-colored liquid is evacuated, followed by about 5 cc. of thick creamy fluid, which comes from the interior of one rib and does not involve the pleura. Cultures are taken from this. The costochondral junctions are soft but not cystic. Transection of the sternum shows deep purplish marrow in the manubrium, but none is identified in the gladiolus or xiphoid process.

Peritoneal Cavity: This contains about 30 cc. of clear fluid; there are a few fine adhesions present, especially around the cecum and the gall-bladder, the operative field about the splenic bed also showing a few. Slightly opaque areas are noted throughout the otherwise smooth and translucent peritoneum. The appendix is in its usual

position. The liver border shows two protuberant lappets separated from each other by a relatively retracted groove; one of them lies 7 cm. below the right costal margin in the midclavicular line, the other 6.5 cm. below the left rim in the midclavicular line, while the groove lies 7 cm. below the xiphoid process. The diaphragm is at the fifth right interspace and the sixth left rib. The mesenteric lymph nodes are moderately enlarged and white, the suprapancreatic group being the largest and having a pink color.

Thorax: The thymus weighs 10 gm. The pericardial cavity contains 50 cc. of watery, blood-tinged fluid in which some clots are found; it is thought that these originate from petechiae in the epicardium. There are no pericardial adhesions. The heart and aorta are not remarkable. The pleural cavities contain about 20 cc. each of clear colorless fluid. Aside from moderate enlargement of the peribronchial lymph nodes the pulmonary apparatus is unremarkable.

Organs of the Neck: The cervical lymph nodes are not much enlarged, the thyroid gland and organs of the neck are not removed, palpation and superficial inspection indicating that they are not abnormal.

Abdominal Organs: The liver weighs 1130 gm. Its lower presenting margin has been described; its consistence is firm, its color a light reddish and its section surface shows a definite increase in the connective tissue, with the formation of large lobules which cause a slight bosselation of the peritoneal surface and are of a light yellow color. Fine fibrous strands separate the masses of liver tissue from one another. The gall-bladder and its ducts are unremarkable. The spleen was removed at operation and has been described elsewhere. The pancreas is quite firm, but not otherwise remarkable. The suprarenal glands and the kidneys and urinary tract are also of the usual appearance, excepting for indistinct marking and pallor of the kidneys. The alimentary tract shows no notable departure from the usual appearance.

Head: The brain is not abnormal and exploration of the internal ears and accessory sinuses is unattended by the finding of anything of note. The skull shows no areas of porosity, or other changes.

Skeleton: The sternum has been described. The lumbar vertebral bodies contain uniformly dark purplish marrow, and after fixation small white spots appear on the section surface. There are cysts in each of the intervertebral cartilages examined, which are apparently

simple, unlined absorption defects (Fig. 2). The right femur contains masses of dark red bone marrow, particularly prominent at its lower end. There are no changes in the cavity of the right knee joint, its surface being everywhere smooth and shining. The left ilium contains no recognizable marrow; its spongiosa is quite dry and leathery.

MICROSCOPIC EXAMINATION

Thymus: There is marked fatty involution of the organ with necrosis and degeneration of the Hassal's corpuscles, which are difficult to find; a parathyroid gland of normal appearance is included in the section.

Heart: Nothing of note is seen.

Lungs: These are free from any definite bronchopneumonic areas and, aside from rather numerous mononuclear cells in the alveoli and scattered areas of interstitial lymphoid infiltration, they are unremarkable. No fat is demonstrable in the alveolar phagocytes.

Liver: The most striking feature in the sections is the large amount of fat in the parenchyma of the organ, globules being generally distributed throughout the section and swelling the liver cells to capacity. This may be slightly more marked on the portal side of the lobules than on the central venous side, but the distinction is not marked. The tissue between the liver cords is edematous and the Kupffer cells numerous and large, but not as prominent as in the case of lipoid histiocytosis. There are none of the crowded, epithelioid mononuclear cells that one observes in Gaucher's disease. Some of the numerous mononuclear phagocytes in the sinuses show a tendency to phagocytose erythrocytes, sharing in the picture of erythrocytic destruction evidenced in the spleen and lymph nodes, but to a far lesser degree. Most of the portal areas are infiltrated by lymphocytes and there is a definite interlobular fibrosis, demonstrable by Mallory's connective tissue stain. Frozen sections stained with Sudan III and Nile blue sulphate confirm the presence of large quantities of neutral fat in the parenchyma, but fail to demonstrate any of this in the Kupffer cells. No lipoids are demonstrable.

Pancreas: There is rather marked fibrosis of the organ with rather large numbers of mononuclear phagocytes in the fibrous tissue.

Suprarenal Glands: Examination of sections from these is negative.

Kidneys: There are the usual changes noted in toxic nephrosis, with numerous fat droplets in the epithelium of the convoluted tubules, which constitutes a lipoid nephrosis.

Mesenteric Lymph Nodes: Those about the pancreas show the most notable lesions (Fig. 3). The sinuses between the secondary follicles are greatly dilated and filled with large numbers of large mononuclear cells, which are actively phagocytosing erythrocytes in enormous numbers and, to a lesser extent, engulfing lymphocytes and other leukocytes. The follicles are compressed into mere ribbons between the greatly dilated sinuses. One section shows cells that are indistinguishable from megakaryocytes. Frozen sections stained with Sudan III and Nile blue sulphate demonstrate a little neutral fat in the capsule of the nodes, but none whatsoever in the phagocytes, which are also free from lipins.

Bone and Marrow: The intervertebral discs exhibit a simple loss of central substance, with a ragged wall to the cysts quite free from any cellular lining or inflammatory reaction. The adjacent vertebrae show a few fine bony trabeculae separated by very large spaces lined with endothelium and loosely packed with erythrocytes. Relatively few phagocytes are found in these sections. In those from the femur, on the contrary, there is considerable phagocytosis of erythrocytes by large mononuclear cells, together with relatively slight erythroblastic and only moderate myeloblastic activity. A few small areas of degenerating polymorphonuclear leukocytes (rather like tiny abscesses) are noted. Plasma cells are encountered in moderate numbers. There is early fibrosis of the marrow and occasional small masses of tangled fibrin are seen here and there. The general picture is reproduced in Figure 4. The sections from the sternum show, in addition, myxomatous degeneration of the marrow. The process in the marrow of these various bones, then, represents chiefly degeneration with a slightly inflammatory appearance, rather than proliferative activity. Supravital stains of the marrow add nothing to the picture.

Skin: No petechial hemorrhages are caught in the sections, but the derma contains many large mononuclear cells and lymphocytes and is edematous.

Special Stains: Sections of the bone marrow, lymph nodes and liver give a positive iron reaction by Perl's method, but the cells show none of the delicate, light blue lines characteristic of Gaucher's dis-

ease. The reaction is in the form of coarse blue granules in the phagocytes and differs in no way from that seen in the neighborhood of hemorrhages, or in cases of hemosiderosis from various causes.

LABORATORY EXAMINATIONS

Bacterial Examinations: Cultures were taken postmortem from the heart's blood and pericardial fluid and showed *Staphylococcus aureus hemolyticus* and a *Streptococcus hemolyticus* in both; others from the pockets over the ribs showed *B. proteus*, while those from the sternum showed *Staphylococcus aureus hemolyticus*. The *B. proteus* was a recent infection, the other two already having been found ante mortem.

Animal Inoculation: Emulsion of the liver was injected into the ear vein of a rabbit and into the peritoneal cavity of a guinea pig, emulsions of bone marrow being similarly inoculated into two other animals. All four were killed 27 and 29 days after inoculation without anything abnormal presenting at autopsy.

SUMMARY AND DIAGNOSES

Autopsy Summary: The outstanding features are: (1) Notable increase in mononuclear phagocytes in the spleen and the lymph nodes of the peritoneal cavity and, to a lesser degree, in the bone marrow. (2) The absence of demonstrable fat or lipins in the phagocytes. (3) Simple cystic degeneration of the intervertebral discs.

Anatomical Diagnoses: Healed splenectomy wound, moderate hyperplasia and hyperemia of the bone marrow, cysts of the intervertebral discs, petechiae in the skin over the trunk and in the epicardium, pericardial effusion, and granular degeneration of the tubules of the kidneys.

DISCUSSION OF THE DIAGNOSES

This case, then, presents the following features already assumed to be pathognomonic of reticuloendotheliosis or aleukemic reticulosis:

Clinical Features: Universal petechial eruption on the skin; secondary anemia; enlarged liver, spleen and lymph nodes; subfebrile temperature; bony changes as determined by X-ray, and epistaxis (Borissowa⁶).

Pathological Features: Universal petechial eruption on the skin, petechiae in the epicardium; destruction of Hassal's corpuscles in the thymus (Letterer,⁷ Guizzetti¹); generalized enlargement of the lymph nodes in certain regions; enlargement and fatty infiltration of the liver; large spleen; sclerotic changes in the liver and spleen, to a lesser extent in the bone marrow; bony changes already mentioned in three cases in the literature (*loc. cit.*) and pathological changes in the marrow. The microscopic picture of the spleen, liver, lymph nodes and marrow accurately corresponds with that described in most of the cases reported. The small necrotic foci over the ribs were also mentioned in one case (Letterer⁷) where there was a reddish phlegmon over a rib. With these facts in our possession it seems justifiable to add to the above anatomical diagnoses the more specific one of "non-lipoid histiocytosis," substituting this for the rather inclusive terms (reticuloendotheliosis, reticulosis, and so on) hitherto used for the same condition. For the same reason, it appears that we are dealing with an entity in the way of a disease and that we are entitled to add this case to those already reported.

DISCUSSION OF THE LITERATURE

Authorities are apparently agreed that the first cases to be published were those of Borissowa,⁶ who described three under the caption "Beiträge zur Kenntnis der Bantischen Krankheit und Splenomegalie." Her findings were in close accord with our present conception of reticuloendotheliosis and decidedly at variance with the picture of an early Banti's syndrome. She described the phagocytosis noted in our case with meticulous accuracy and it is a pity that our present methods of tissue examination were not at her disposal thirty years ago when she wrote her paper.

As we have stated, there are two schools of thought as to the nature of this disease: the inflammatory and the leukemic. Hittmair⁸ would extend it to cover infectious mononucleosis at the one end and diffuse sarcomatosis of the retothelial type at the other. Some authors consider it to be a sort of leukemia in which there may be three phases: the aleukemic (as typified by our case), the subleukemic, and the leukemic as represented by Swirschewskaja⁹ in her report in which 95 per cent of atypical mononuclear cells were circulating in the blood. Richter¹⁰ reported a case in which there was

a general picture closely resembling reticuloendotheliosis, together with a typical lymphoid leukemia. On the other hand, such authors as Letterer,⁷ Schultz, Wermbter and Puhl,³ Krahn,¹¹ Akiba,¹² Goldschmid and Isaac,¹³ and others, believe it to be of an infectious nature, a response to some low grade stimulus of a bacterial nature. Wihman¹⁴ is one of those who will not commit himself as to the probable etiology.

The presence of subfebrile temperature in many cases, the history of some sort of infection preceding the development of symptoms, the similarity of the reaction to that often seen locally in lymph nodes draining chronic foci of inflammation or operative sites,—all these lead one seriously to consider an infectious basis for the condition, however alluring may be the conception that one is dealing with a third type of leukemia. Against the latter is the fact that there is a definite lack of anaplasia in the type cells (such as is seen in the true leukemias or in retothelial sarcomatosis), little evidence of mitotic activity and the lack of truly proliferative foci and immature forms of the type cells in the blood. One cannot overlook the fact that there is a vague similarity in the pictures of the lymph nodes and spleen to those seen in myelogenous chloroma or the localized myeloid tumors of myelogenous leukemia, but such areas are not found outside of the lymphoid apparatus in this non-lipoid histiocytosis, whereas they are almost the rule in the parenchyma of various organs in chloroma. Cases of true, generalized retothelial sarcomatosis are sometimes encountered and these have a totally different appearance from that of the disease under discussion, as well as a far more lawless distribution. Roulet¹⁵ makes this comparison clear in his article on retothelial sarcoma, reporting a case similar to ours for contrast. Lack of invasion of epithelial or other tissue foreign to the reticuloendothelial system in such cases is perhaps the most telling point in favor of inflammatory origin, and seems to mark it as a response to toxic stimuli rather than a form of neoplasia.

We have quoted a number of articles in the body of this paper; there are others that may be referred to if the reader desires to pursue the subject. Perhaps the most complete bibliography is given by Hittmair,⁸ who has reported a number of cases and made an extensive study of the disease. Besides those already referred to there are interesting articles by Tschistowitsch and Bykowa,¹⁶ Ugriumow,¹⁷ Ewald,¹⁸ Benecke,¹⁹ and Ungar.²⁰ Benecke's article deals with a case

of retothelial sarcomatosis, while Ungar's showed both a retothelial sarcoma of the humerus (with metastases) and the histiocytic hyperplasia of the disease under discussion. Both of these papers are interesting in connection with Roulet's two publications.

It is not our intention to discuss this question from the clinical standpoint; we have given as much clinical history as we deemed necessary for clarity and will leave its elaboration to our colleagues in the department of pediatrics. Our aim is to bring the case to the notice of American pathologists in the hope that the disease may be more readily recognized in this country and, perhaps, satisfactorily explained and placed on a more solid footing.

Microscopic sections of the frontal area of the brain were examined in silver impregnations after this paper was submitted for publication. They show a surprising increase in the number of microglia cells in the area examined, as well as an increased number of rod cells. Compound granular corpuscles were not found. The picture offered a striking similarity to that of general paresis and one quite unusual in so young a subject.

This examination was carried out by Dr. Lewis D. Stevenson, neuropathologist to the college.

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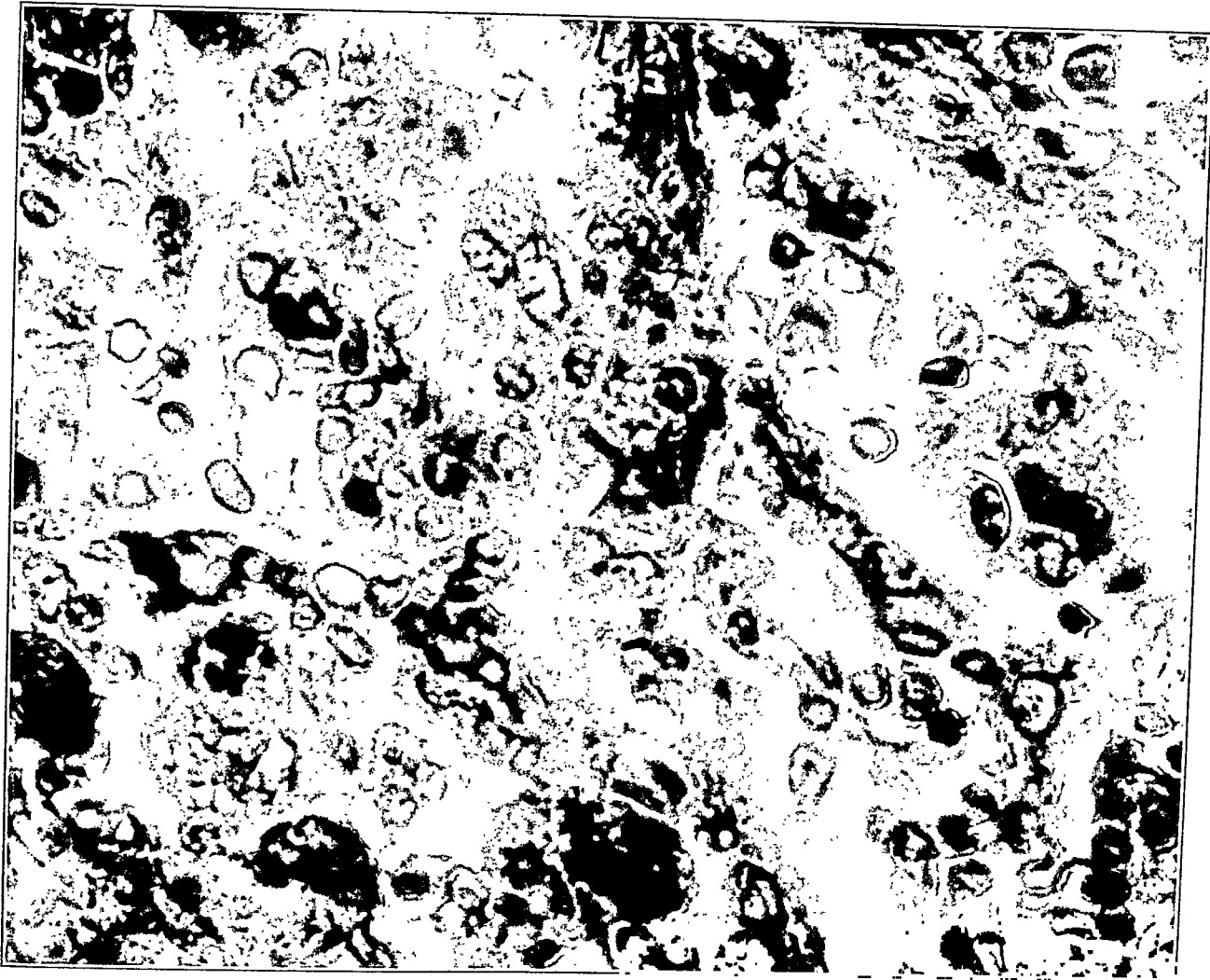
DESCRIPTION OF PLATES

PLATE 47

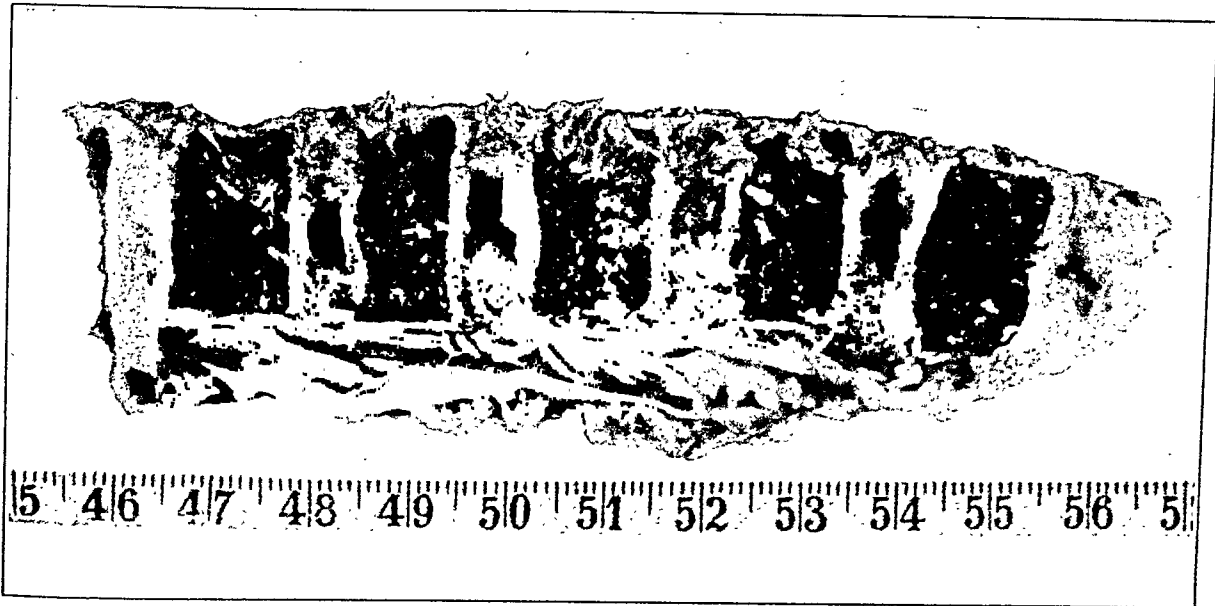
The photographs were made by Mr. William Dunn of the department of photography, Cornell University Medical College.

FIG. 1. Photomicrograph of the spleen. The erythrocytes are deeply stained and photograph black in most instances. Note the marked phagocytosis of these and the wiping out of the normal splenic topography by the masses of proliferated retothelial cells. Masson trichrome anilin blue. $\times 790$.

FIG. 2. Photograph of the excised lumbar segment of vertebral column showing the cystic degeneration of the intervertebral discs and the whitish spots in the marrow of the vertebral bodies.



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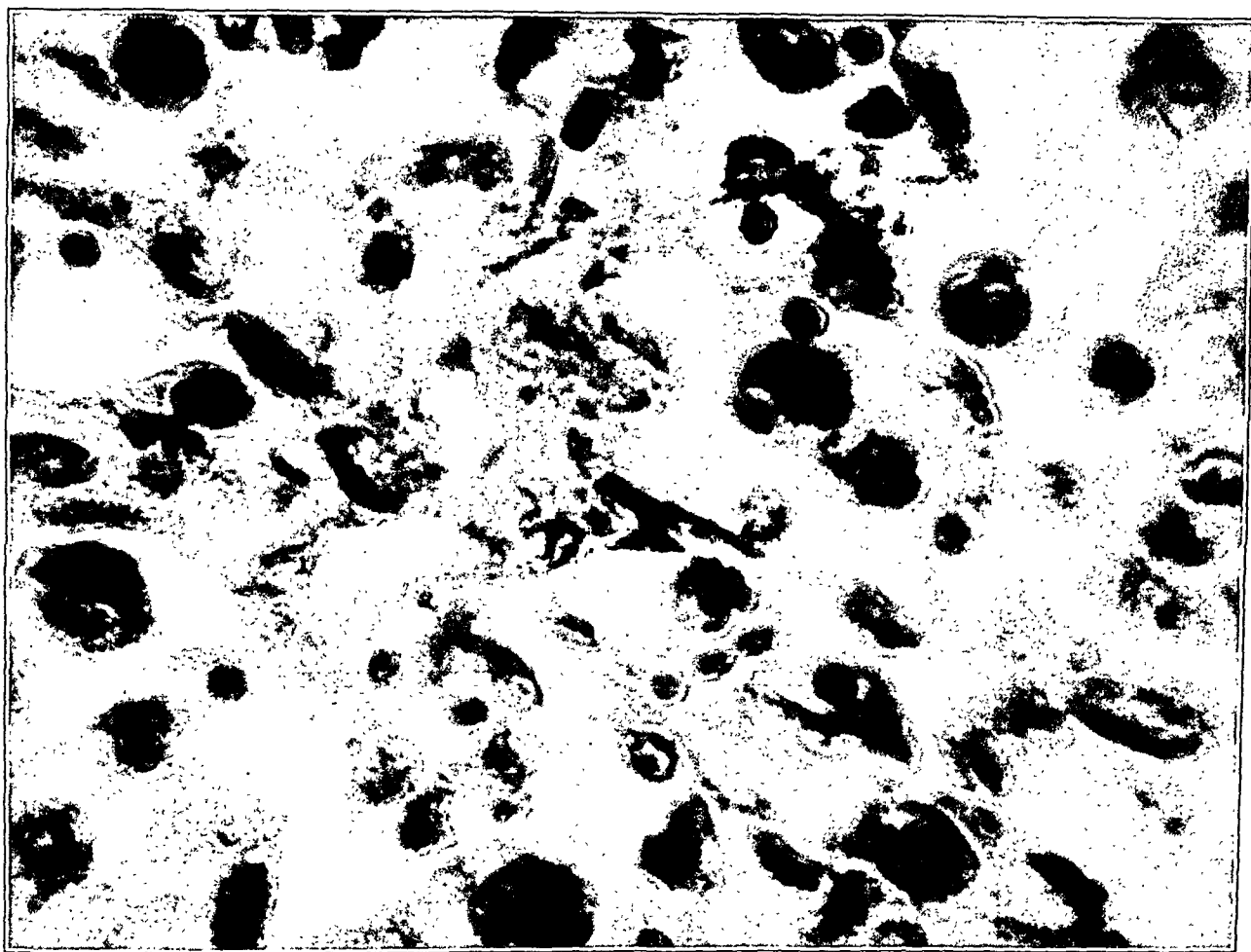


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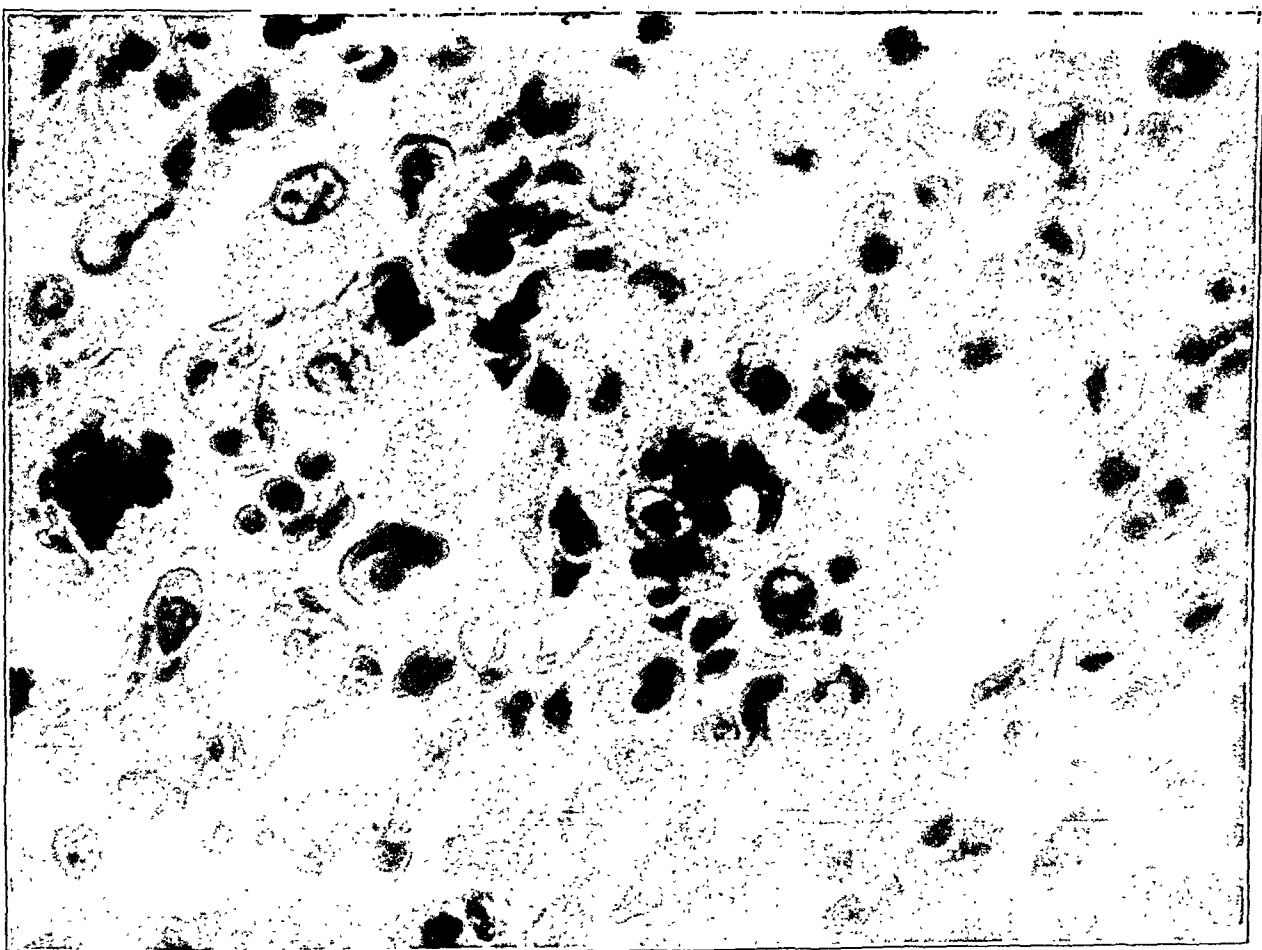
PLATE 48

FIG. 3. Photomicrograph of abdominal lymph node. The dilatation of the sinuses and the presence in them of phagocytes actively engulfing erythrocytes is very noticeable. Masson trichrome light green stain. $\times 350$.

FIG. 4. Photomicrograph of the bone marrow of the femur. Phagocytosis is a feature here, although less marked than it is in the preceding figures. The topography of the marrow is much altered by the presence of numerous macrophages. Masson trichrome light green stain. $\times 790$.



3



4

MULTIPLE BRANCHIOGENIC ACANTHOMA *

REPORT OF A CASE

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While acanthomas arising in branchial cysts are well recognized and not especially rare (we have seen 2 other cases, probably branchiogenic acanthoma, and 14 branchial cysts among some 2000 cysts and tumors of all kinds in this laboratory), it is believed that the occurrence of 3 such tumors in one patient in the space of 3 years merits reporting, especially as the tumors in question all arose definitely in cysts in different locations. Warren and Gates¹ list many cases of multiple acanthoma, among which are 4 in which 1 of the tumors was located in the neck. These were classed as cutaneous rather than branchiogenic, and there were no cases of multiple acanthoma of the neck, either cutaneous or branchiogenic.

The patient, a white seaman, aged 55 years, applied for treatment on April 28, 1930, for a small indurated ulcer of the lower lip. The ulcer was of about 3 weeks duration, and the indurated area was about 1 cm. in diameter. There was also a hard tender gland about 2 by 2.5 cm. in the left submaxillary region.

At operation at the United States Marine Hospital at Port Townsend, Washington, the lesion on the lower lip and a cyst about 2 by 2.5 cm. was removed from the left submaxillary region. The cyst ruptured in the course of removal. Its contents were sebaceous in character.

On Aug. 14, 1930, the patient returned to the United States Marine Hospital at Port Townsend, complaining of a lump of 5 weeks duration in the right submaxillary region. There was no evidence of recurrence of the lip lesion or of the tumor in the left submaxillary region. A second cyst was removed from the right submaxillary region, also about the same size as the first, and containing sebaceous material.

* Received for publication May 1, 1933.

After this the patient returned to sea and was not seen again until he applied for treatment at the United States Marine Hospital in Seattle, Washington, on March 1, 1933, complaining of a lump under his chin of 3 months duration. At operation a cyst about 3 cm. in diameter, containing necrotic material, not attached to the overlying skin, lying in the midline against the under surface of the symphysis of the mandible was found. A fragment of periosteum was removed with the cyst but the underlying bone was smooth.

The material received for histological examination consisted of the lip lesion and the three cysts mentioned above. Abstracts of the reports made are as follows:

"The lip shows only moderate downgrowth of the epithelium, with a few poorly limited prickle cell strands and an occasional pearl. The corium shows rather dense round cell infiltration and a few foreign body tubercles." The cyst from the left submaxillary region excised April 30, 1930, showed "an infiltrating tumor composed of cords and strands of prickle cells, with keratinized pearls and keratinizing small multilocular cysts. In one area this tumor is definitely invading a lymph gland."

The second cyst, removed on Aug. 15, 1930, from the right submaxillary region, presented "a cyst wall lined by a relatively well limited stratified squamous epithelium, and about this a malignant newgrowth composed of masses of epithelial cells with well defined prickles, cells with keratohyaline granules and keratinized pearls." Marked infiltrative tendencies and filling of lymphatic spaces were noted, mitoses were few, and well marked round cell infiltration of the stroma was noted. Adjacent scar tissue, fat, fasciae, muscle and hyperplastic lymph glands were not invaded.

The third cyst, removed piece-meal from the symphysis of the mandible on March 10, 1933, showed a multilocular structure. There were several small cysts filled with necrotic débris and lined or partly filled by foreign body granulation tissue and granulomas. There was a small lymph follicle in the wall of one of the cysts. The surrounding areolar, fascial and muscular tissues were fibrotic, infiltrated by lymphocytes and diffusely and irregularly invaded by strands and masses of poorly limited prickle cell epithelium with concentric keratinizing cell nests. Mitoses were moderately numerous. A small lymph gland and part of the excised muscle were not invaded.

The lip tumor was diagnosed as early epithelioma (Grade I); the

three tumors arising in cysts were all considered as definitely malignant and of about the same grade (Grade III). The first two appear to have arisen in cysts derived from the second branchial cleft; the last, lying in the submental region, probably represents a malignant transformation of a mesobranial cyst. That these tumors represent independent primary growths is indicated by their grossly cystic nature and sebaceous contents.²

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BENZOL POISONING WITH HYPERPLASIA OF THE BONE MARROW *

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Chronic benzol poisoning is recognized as a potential cause of fatal leukopenic anemia, accompanied by aplasia of the bone marrow. Hamilton ¹ in her recent review was able to collect only 35 more or less complete autopsy reports on cases of benzol poisoning. In 2 of these (Cabot,² and Martland, quoted by Hamilton,¹) there was hyperplasia instead of aplasia of the bone marrow, although the blood picture did not differ in essential respects from the other cases. There has been some doubt as to whether or not the presence of bone marrow hyperplasia is compatible with the diagnosis of chronic benzol poisoning, and Hamilton left the question open for want of evidence. Thompson, Richter, and Edsall ³ have called attention to the frequency with which the blood picture of aplastic anemia is associated with marrow hyperplasia. A brief description of the following case is included in their paper, but the case was of such interest that it seemed advisable to report it in detail.

REPORT OF CASE

Clinical History: Presbyterian Hospital Case No. 364,427. E. B., an American-born, white male clerk, 53 years of age, entered the hospital on Dec. 29, 1932, with the complaint of weakness and bleeding from the nose and gums for 2 years. His family history was negative in regard to blood dyscrasias and was otherwise irrelevant. His past history was essentially negative, except for moderate nocturia for the previous 10 years, and for occasional nosebleeds since boyhood. These had lasted about 15 minutes and had occurred at intervals of several months. Later questioning revealed the fact that he was an amateur photographer and for 4 to 5 months prior to onset of illness had used benzol for cleaning bromoil brushes 3 or 4 nights a week in a closed room, without taking any precautions. Further inquiry revealed that the purchases of benzol began 5 years before entry to the hospital. The patient's family also stated that he had used benzol to remove the paint from the floors of his home. There was no exposure to it during the past year and one-half.

The patient's illness began 2 years before admission with a severe nose-bleed lasting 18 hours, and finally controlled with adrenalin. His gums began bleeding at the same time. His physician kept him on a diet excluding red meat.

* Received for publication July 1, 1933.

TABLE I
Blood Counts

Date	Hemo- globin	Red blood cells	Reticulo- cytes	Normo- blasts per 100 white blood cells	White blood cells	Neutro- phils	Eosino- phils	Basophils	Lympho- cytes	Mono- cytes	Myelo- blasts and myelo- cytes	Unclassi- fied	Platelets
5/16/32...	per cent 48	2,650,000	..	3	1800	53	46	1
7/13/32...	65,000
7/9/32....	40	2,480,000	2000	41	1	..	56	2
7/23/32...	28	1,600,000	2800	37	..	2	49	12
8/1/32....	40	2,030,000	1000	28	68	4	30,000
8/8/32....	40	2,310,000	1800	32	64	4	14,000
8/15/32...	20	2,200,000	3100	19	79	2	14,000
8/24/32...	32	1,560,000	1500	48	50	1	1
9/10/32...	37	1,700,000	..	10	2200	59	0	..	39	1	1	..	6,400
12/29/32...	55	2,370,000	1800	52	44	2	2	..	18,000
12/31/32...	55	2,500,000	..	10	2200	28	1	..	66	5
1/6/33....	45	2,500,000	12.3	2	1700	42	56	2	18,000
1/16/33...	35	1,700,000	10.0	5	1400	66	30	4	21,000
1/18/33...	37	1,176,000	1.04	..	825	59	38	1	2	..	16,000
1/10/33...	35	1,170,000	4.1	1	1100	47	52	1
1/20/33...	32	1,302,000	0.2	5	1190	33	58	9	14,000
1/21/33...	3150	34	0.5	..	51	6	..	7.5	..

Until Aug. 24, 1932 the counts were done by Dr. E. J. Buxbaum of Jamaica, New York. The count of September 10 was done in the Shiel Laboratory, Queens Village, New York. After admission on Dec. 29, 1932, the reports are from the Presbyterian Hospital laboratories.

One year later he had a carbuncle on the nose, which healed. In May, 1932, the first blood count was done and he was found to be anemic (see Table I). A course of liver extract and liver diet was prescribed at this time. During July, 1932, he received four transfusions without improvement in the blood picture (see Table I). He felt fairly well but gradually lost strength.

Physical Examination: Temperature 100.8° F, pulse 84, respirations 20, blood pressure 150/80. The patient was a well nourished man with a sallow complexion and dark brown pigmentation of the skin over the hands and ankles. The eyes were normal, except for mild sclerosis of the retinal vessels and a few patches resembling old hemorrhages in the fundi. The gums, teeth and tongue were somewhat coated and deeply stained. The heart and lungs were normal. The abdomen was distended with a palpable spleen and liver. The regional lymph nodes were not enlarged.

Laboratory Data: The blood counts are given in Table I. The red cells showed marked variations in size and shape with polychromatophilia and

TABLE II

Polymorphonuclear Counts (Method of Cooke and Ponder)

No. of lobes	I	II	III	IV	V	Total white blood cells
Normal	10	25	47	16	2	
Jan. 16, 1933	26	56	18	0	0	1400
Jan. 19, 1933	38	52	8	2	0	1100

basophilic stippling. Two normoblasts were seen. The polymorphonuclear cells were young. Polymorphonuclear counts were made on January 16th and 19th, after the method of Cooke and Ponder.⁴ Fifty polymorphonuclear leukocytes were counted each time and a marked shift to the left was found, as is shown in Table II.

Bleeding time 2 hours, coagulation time 12.5 minutes, no clot retraction within 2 hours. Fragility test: hemolysis begins — patient 0.425, control 0.45; complete — patient 0.30, control 0.325. The blood Wassermann test was negative. Serum bilirubin: very faint trace. Blood chemistry: cholesterol 0.14 per cent, uric acid 3 gm. per 100 cc., serum protein 5.2 per cent, blood sugar 1.79 gm. per 1000 cc., serum calcium 11.1 mg. per 100 cc., serum phosphorus 3.3 mg. per 100 cc., blood non-protein nitrogen 22.1 mg. per 100 cc. Total plasma proteins 4.68 per cent, albumin 2.25 per cent, globulin 2.07 per cent, fibrinogen 0.36 per cent.

Urine: specific gravity 1.018, very faint trace of albumin on one occasion, otherwise negative. Stool: dark olive green, soft, guaiac 4 plus.

Course of Illness: The temperature was septic in type, ranging from 99.6° to 101.2° until the last week of life, when it rose irregularly to 104° F. The patient received the following treatment: A series of intramuscular injections of Lilly's liver extract, an intramuscular injection of 22 cc. of whole blood, three direct transfusions with a total of 1600 cc. of blood, and subcutaneous injections of anti-snake venom. Two days before death he was given 5 cc. of nucleotide intramuscularly. The gums continued to ooze and the nose to bleed to some extent. On January 17th he had a hemorrhage into the intestinal tract and

there was a bloody stool on the following morning. On January 18th he developed dyspnea and râles at the bases of both lungs. On January 19th he had a pain in the left side, which was thought to be a splenic infarct, and fresh petechial hemorrhages. A blood culture taken on January 20th showed *Streptococcus hemolyticus*. Death took place from respiratory failure Jan. 22, 1933.

POSTMORTEM EXAMINATION

Autopsy No. 11,143, 12½ hours postmortem.

Gross Examination: The body is 175 cm. in length. The lips and nail beds are pale. Purpuric spots varying from pin-point size to 1 cm. in diameter are scattered over the body, but are more numerous over the thorax and abdomen and on the inner surfaces of the arms and thighs. They are not sharply outlined, are often irregular and vary in color. The feet, ankles, hands, and to some extent the face, are brownish in color without scaling or thickening. The sclerae have a faintly icteric tinge. The nose and ears are negative. The gums are purplish and spongy, with blood clots streaking over them, but without obvious ulcerations. The tongue is dry and black. The remainder of the external examination is essentially negative.

The abdominal subcutaneous fat is 5 cm. in thickness and contains many small hemorrhages. The peritoneal cavity contains 75 cc. of slightly cloudy, blood-tinged serous fluid. The right thoracic cavity contains 200 cc. and the left 300 cc. of a fluid similar to that in the peritoneal cavity, but they are not otherwise remarkable. There is a small mass of reddish, thickened connective tissue behind the aorta at the level of the diaphragm.

Heart: weight 540 gm. The enlargement is symmetrical and petechial hemorrhages are found in the epicardium, myocardium and endocardium. A very slight thickening and roughening in a small area of the anterior leaflet of the tricuspid valve is present. The valves are otherwise normal. There are a few sclerotic plaques in all the coronary vessels, which do not occlude the lumina.

Aorta: Normal.

Lungs: The right lung weighs 870 gm., the left 800 gm. Many small, subpleural hemorrhages are seen. The posterior two-thirds of all lobes of both lungs are firm, non-crepitant, mottled and moist. A small caseous nodule lies near the main bronchus of the right lower lobe.

Spleen: Weight 370 gm. Measurements 15 by 9.5 by 4.5 cm. The margins are rounded and the cut surface bulges above the capsule

and is moist. The pulp is soft but not friable, and contains many hemorrhagic areas. The malpighian corpuscles are distinguished with difficulty. No infarcts are found.

Liver: Weight 2580 gm. Measurements 28 by 19 by 10 cm. The organ is pale and the shape normal. It cuts with ease, is softer than usual, cut surface being moist and bulging above the capsule. The lobular pattern is distinct; the central areas are red, the portal areas form wider yellow zones. The bile passages are normal.

Pancreas: Normal.

Adrenals: Large, soft and friable. The cortex is gray with occasional small yellow nodules, and the lipoid content appears extremely small. There are many small, fresh hemorrhages in the adjacent fat.

Kidneys: The right kidney weighs 240 gm. and measures 13 by 7 by 3.5 cm. Both kidneys are normal, except for pallor and for many small hemorrhages in the pelvic fat and mucosa and a few in the kidney parenchyma. The left kidney weighs 240 gm. and resembles the right.

Pelvic Organs: The bladder is much dilated and hypertrophied and there are many small hemorrhages in the mucosa, the largest measuring 0.5 mm. in diameter. There is some prostatic hypertrophy. The testes and seminal vesicles are normal.

Gastro-Intestinal Tract: The stomach is much dilated and there are innumerable small hemorrhages in the mucosa, many of which have formed ulcers. These hemorrhages are also found in the first centimeter of the duodenum, but are not found in the remaining duodenum and small intestine. Similar hemorrhages are found in the cecum and to a less extent in the rest of the colon.

Neck Organs: Hemorrhages are present in the mucosa of the pharynx and the base of the tongue, and there is marked edema of the glottis. The *thyroid* is normal. The *lymph nodes* of the neck and axilla are somewhat enlarged, firm and bright red. The other lymph nodes are normal. Smears of the *sphenoid sinus* show great numbers of streptococci.

Bone Marrow: This was obtained from the sternum, the fifth rib, the lumbar vertebrae, and the femur at a point between the middle and lower thirds. It is similar in all these places, and is semifluid, pale red, and without fat. In the femur marrow there are a few hemorrhages.

Brain: Normal except for a few pial hemorrhages.

MICROSCOPIC EXAMINATION

The changes of greatest interest are found in the spleen, liver and bone marrow.

Spleen: There are many hemorrhages in the pulp. The malpighian corpuscles have tortuous and hyalinized central vessels and are edematous. Around the margins of the corpuscles there are many myeloid cells. Most of the abnormal cells have large, hyperchromatic, irregularly shaped nuclei surrounded by a little faintly basophilic cytoplasm. A few mature polymorphonuclears are found. The sinusoids are wide, with prominent endothelium, and they contain many clumps of nucleated red cells and megaloblasts. A few vessels are seen to be plugged with bacteria. Many of the cells in the pulp and a few in the corpuscles contain granules which stain for oxidase, and are especially well brought out by the dopa stain. The azure eosin stain reveals neutrophilic granules in most of the abnormal cells (Fig. 3). Phagocytes in the pulp contain much iron pigment.

Liver: The portions of the lobules around the efferent venules show extensive degeneration, often with complete loss of liver cells and with extensive hemorrhages. In these areas there are many polymorphonuclear leukocytes with multilobulated pyknotic nuclei. There are small numbers of myelocytes in the portal areas. The sinusoids contain many nucleated cells, some of which are normoblasts; others are myelocytes. A few sinusoids contain bacterial emboli. There is a little pigment in the Kupffer cells, which takes the iron stain. Most of the cells in the central half of each lobule and in the portal areas contain granules that take the oxidase stain (Fig. 4).

Lymph Nodes: The cervical lymph node contains many hemorrhages and many myelocytes. There are a few clusters of foreign body giant cells. The axillary lymph node is similar. The dopa and alphanaphthol pyronin peroxidase stains show that most of the myeloid cells are in the sinuses, though a few are in the pulp.

Bone Marrow: Marrow from the femur, rib, sternum and lumbar vertebrae is alike. All fatty marrow is replaced by blood cell formation. Many small islands of normoblasts are seen. Mature cells of the myeloid series are rare, but there are many nests of premyelocytes, cells with a large vesicular nucleus containing nucleoli and a pale pink, finely granular vacuolated cytoplasm (Fig. 3). Myeloblasts are few. Occasional small megalokaryocytes are found.

Many large phagocytes are stuffed with fragments of red cells and other débris. The azure eosin stain shows that the majority of the mononuclear cells contain neutrophilic granules (Fig. 4). Most of the cells give a positive reaction to the dopa stain and to the alphanaphthol pyronin peroxidase stain. Sections through the malleus incus and the setrous portion of the temporal bone show a similar marrow.

Other Organs: There is a recent bacterial vegetation on the tricuspid valve consisting of streptococci. Abscesses are found in the tonsil, epiglottis and behind the aorta at the level of the diaphragm. Bacterial emboli and hemorrhages are present in all the organs. There is an early bronchopneumonia, with very few polymorphonuclears in the exudate. The adrenals have undergone infectious degeneration. There are a few tubercles in the cervical and bronchial lymph nodes. The prostate is hypertrophied.

Anatomical Diagnoses: Aplastic anemia due to benzol poisoning; hyperplasia of bone marrow; hemorrhages in skin, subcutaneous tissue, heart, lungs, spleen, adrenals, kidneys, bladder and meninges; central necroses of liver; hemosiderosis of liver and spleen; acute sphenoiditis; acute tonsillitis; acute pharyngitis; abscess of epiglottis; acute bacterial endocarditis, tricuspid valve (*Streptococcus hemolyticus*); bacteremia (*Streptococcus hemolyticus*) with metastases; acute splenic tumor; infectious degeneration of adrenals; acute suppurative nephritis; erosions of stomach; bilateral hydrothorax; ascites; edema of meninges; lobular pneumonia; cardiac hypertrophy; benign hypertrophy of prostate; tuberculosis of cervical and bronchial lymph nodes.

DISCUSSION

The essential features of this case are the same as in the 2 previously reported ones, namely, prolonged exposure to benzol, a leukopenic anemia, and active formation of both erythrocytes and leukocytes in the bone marrow, giving a picture suggestive of that in pernicious anemia. In Martland's case there were areas of both erythrocyte and leukocyte regeneration in the liver, and in Cabot's case there was erythropoiesis in the spleen and slight cirrhosis of the liver. In Cabot's case, which is reported in more detail, other points of similarity are present. The blood contained reticulocytes and normoblasts, in spite of the severe anemia with a hemoglobin of 35 to

50 per cent. The leukopenia of 2000–3000 white blood cells was due chiefly to a decrease of polymorphonuclear leukocytes. Platelets were much diminished and the bleeding and clotting time was prolonged. There was no clot retraction. Bleeding and spongy gums, petechiae in the mucous membranes and congestion of the liver, spleen and kidneys were present. It is perhaps worthy of notice that both of these patients were occupied in the production of artificial leather.

It is interesting to compare these 3 cases with the 2 reported cases of leukemia following exposure to benzol. The case of Delore and Borgomano⁵ was that of a man of 41 years, who was exposed to benzol for 5 years in the process of preparing pyramidon. He developed hematemesis and was found to have multiple hemorrhages of the subcutaneous tissue, hematuria, and spongy and bleeding gums. The lymph nodes were enlarged. Examination of the blood revealed the following: hemoglobin 70 per cent, red blood cells 3,147,000, white blood cells 542,500, lymphoblasts 80 per cent, neutrophilic myelocytes 8 per cent, eosinophilic myelocytes 2 per cent, basophilic myelocytes 1 per cent, polymorphonuclears 6 per cent, lymphocytes 3 per cent. A few normoblasts were present. There was no clot retraction. Death followed gangrenous stomatitis. The autopsy revealed extensive hemorrhages in most of the organs, necrotic areas in the liver, and a large pale spleen. The bone marrow was not described. In view of the many myelocytes and few lymphocytes in the blood, and the difficulty of distinguishing lymphoblasts from myeloblasts, it is probable that this was a case of acute myeloid leukemia. It is impossible to be certain that the leukemia was the result of the exposure to benzol, but the circumstances are suggestive.

The second case, reported by Weil,⁶ was that of a woman of 62 years who worked in the rubber industry. For about 2 years she had had fatigue, anemia, epigastric pain and loss of weight. On examination the essential findings were jaundice, a very large liver, and a spleen that extended below the umbilicus. The lymph nodes were not palpable. The red count was 1,080,000, hemoglobin 30 per cent, without anisocytosis, polychromatophilia, poikilocytosis, or nucleated red cells. The white count was 25,900, polymorphonuclears 60 per cent, lymphocytes 0.2 per cent, middle sized monocytes 32 per cent, large mononuclear cells 2 per cent, neutrophils 4 per cent.

After a series of transfusions and treatment with liver extract and X-rays, the red count rose to 1,240,000 hemoglobin, 40 per cent, and the white count dropped to 5600, polymorphonuclears and myelocytes to 81 per cent. The autopsy revealed a spleen and bone marrow with the lesions of myeloid leukemia. The bone marrow contained many large cells with a single pale nucleus containing nucleoli, and a scanty non-granular basophilic cytoplasm. Many megalokaryocytes, but few nucleated red cells were present. The splenic pulp contained masses of young myeloid cells and some megalokaryocytes. The liver was fatty. There was no evidence of myeloid cell accumulations in either the liver or spleen. There were no hemorrhages.

This case differs from the previous one in the absence of hemorrhages and in the greater maturity of the cells in the blood.

In view of Hamilton's ¹ recent comprehensive review of the experimental work on benzol poisoning only a few of the most pertinent experiments will be mentioned. Selling ⁷ reported regeneration following aplasia in the marrow. Brandino ⁸ (quoted by Hamilton ¹) found complete regeneration of the cells in the marrows and spleens of dogs and rabbits after cessation of treatment with benzol. The recent attempts of Lignac ⁹ to produce leukemia in mice by means of benzol injections are of especial interest. After a number of experiments with various doses of benzol he injected a series of mice with minute amounts of benzol in olive oil once a week for periods of 4 to 10 months, and of the 54 mice used 8 developed some form of leukemia, and 2 more had large lymphoid nodules in various organs without change in the blood picture. An interval of 2 weeks to 2 months intervened between the last injection and death. Of the 8 leukemias, 3 were diagnosed lymphosarcomatosis; 1, lymphoblastic leukemia; 2, mast-cell leukemia; 2, aleukemic myelosis; and 1, eosinophilic myeloid leukemia. It seems discreet to reserve judgment as to the significance of these findings because of the frequent occurrence of various diseases of the lymphoid tissues in mice, and the fact that no mention was made of genetic control of the strain of mice. However, 3 or 4 of the 8 cases were types of leukemia that occur only rarely as spontaneous diseases in mice.

These experiments do not throw much light on the series of cases that have been discussed, except that they show that some degree of hyperplasia of the blood-forming tissues may occur after exposure to benzol. The work of Lignac suggests that hyperplasia may be the

result of repeated minute doses of benzol, but this is not true of any of the 5 cases discussed, for all were exposed to rather large amounts of benzol. It is conceivable that there may be some connection between the blood cell hyperplasia occasionally resulting from benzol and the occurrence of neoplasms as the result of the administration of coal tar. There is further evidence for this view in the experiments of Thomsen and Engelbreth-Holm,¹⁰ who injected tar into the bone marrow of 62 chickens every 5 days for 2 to 3 months and found myeloid hyperplasia in 9 of them, and a blood picture resembling that found in spontaneous chicken myeloid leukemia in 1. This concept, however, must await further confirmation of the existence of leukemia resulting from benzol, since the occurrence of leukemia has not been proved to be more than coincidence, either in the 2 clinical cases or in Lignac's mice.

SUMMARY

A case of benzol poisoning has been reported in which there was progressive diminution of the cellular elements of the blood, but in which the blood contained young cells and the bone marrow was extremely hyperplastic. Two other similar cases have been collected from the literature, together with 2 cases of leukemia associated with exposure to benzol.

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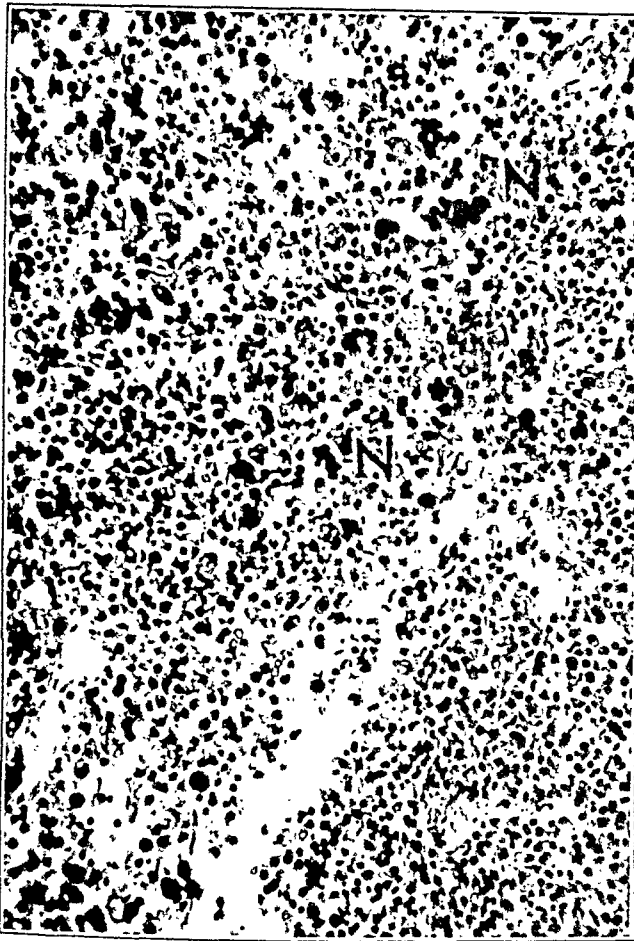
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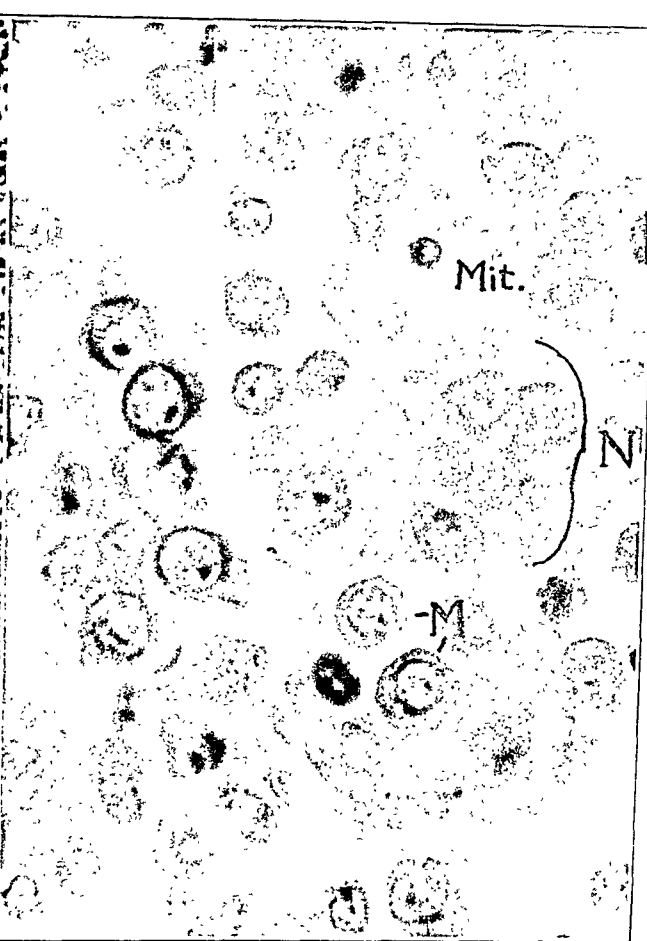
DESCRIPTION OF PLATE

PLATE 49

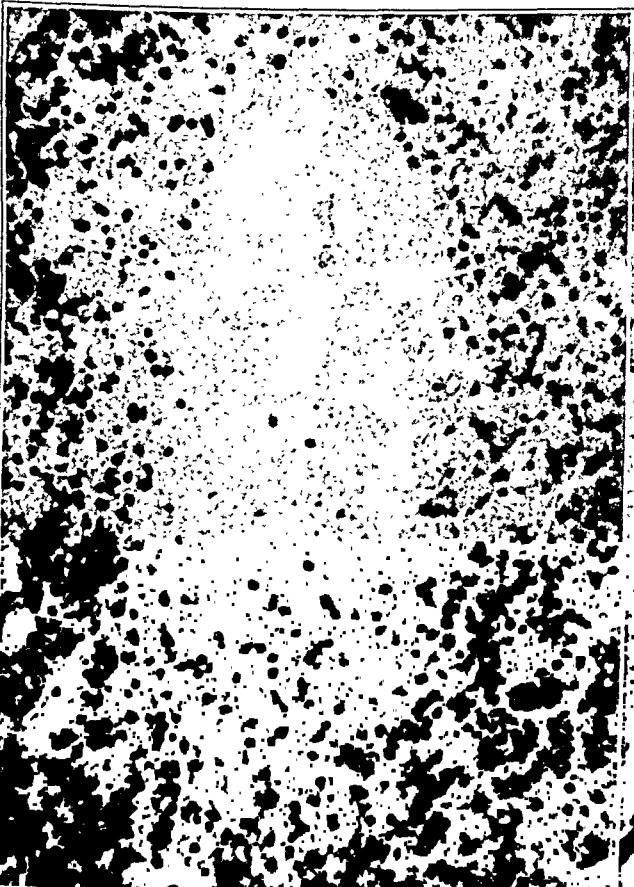
- FIG. 1. Bone marrow (femur). Among the masses of myeloblasts there are many nests of normoblasts (N). Azure eosin stain. $\times 160$.
- FIG. 2. Bone marrow (femur). This shows many myeloblasts (M), one of which is in mitosis (Mit) and one nest of normoblasts (N). Azure eosin stain. $\times 1050$.
- FIG. 3. Spleen, dopa reaction, showing the oxidase-containing cells around a malpighian corpuscle. $\times 160$.
- FIG. 4. Liver, dopa reaction. There are masses of myeloid cells in the portal spaces (P) and somewhat less concentrated groups of them in the central areas (C). A few cells in the sinuses also give a positive reaction. $\times 28$.



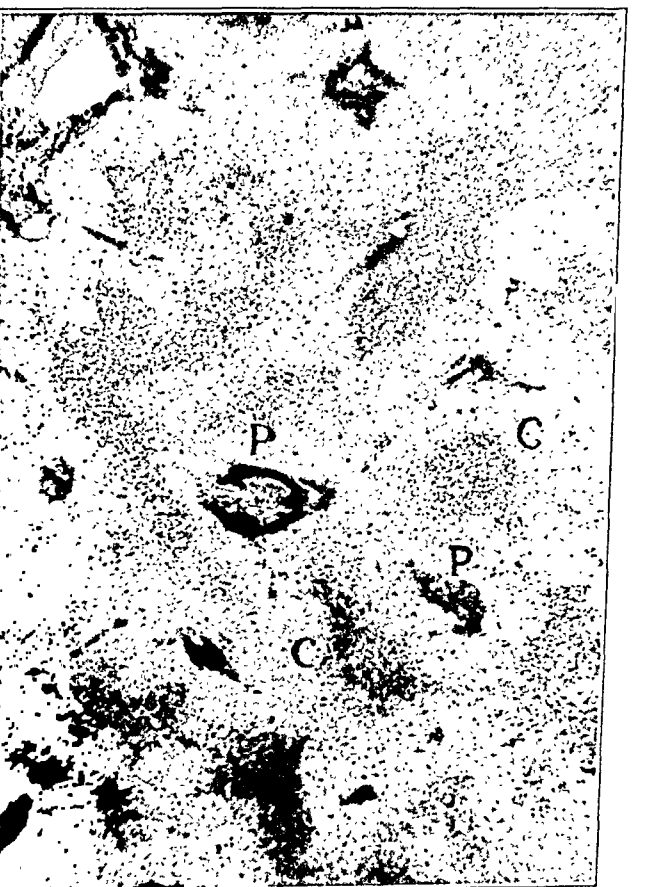
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FRIEDREICH'S ATAXIA *

A CLINICAL AND PATHOLOGICAL STUDY

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The symptom-complex now known as Friedreich's ataxia was first described in 1861 as a slowly progressive weakness and ataxia developing during childhood and gradually advancing from below upward, attacking successively the legs, trunk and arms. Frequently more than one member of the same family is affected. It is further characterized by slow scanning speech, abolition of deep reflexes, scoliosis, foot deformities, integrity of the cutaneous sensations and absence of genito-urinary disturbances. Although many variations have been reported in the minor clinical features, the major symptoms are sufficiently constant to establish Friedreich's ataxia as a definite spinal affection.

The literature was reviewed by Barrett in 1927, from both clinical and pathological viewpoints. Since then numerous clinical histories have been published, but very few pathological studies are reported. In view of the long duration of the disease it is not surprising that pathological studies are infrequent. We have recently had the opportunity to study two cases of Friedreich's ataxia in sisters, one of whom died of an upper respiratory infection and came to autopsy. A detailed histological study of the nervous system was made.

CASE REPORTS

CASE 1. *Clinical History:* A. C., female, 21 years of age at the time of death, had been afflicted with this disease since the age of 5 years, and had been unable to walk since the age of 11 years.

Her early childhood presented nothing of particular significance. She was a full term baby delivered with forceps after a prolonged dry labor. However, there were no head injuries nor was there any respiratory difficulty. The birth weight was 9½ pounds. She was breast fed for 14 months, gained weight steadily and developed normally. Her first teeth appeared at 4 months, she sat up alone at 5 months, talked at 10 months, and walked at 18 months. She had the usual infectious diseases of childhood — pertussis at 9 months, chickenpox at 2 years, measles at 5 years, mumps at 7 years, influenza at 8 years, and tonsillitis almost every winter. She was vaccinated at the age of 8.

* Received for publication August 3, 1933.

When 5 years old the first difficulty in walking was noticed — a certain weakness of the legs and slowness of the movements. Her ankles seemed to twist, her knees would weaken and give way and she would fall frequently; however, she never hurt herself in falling, was never unconscious and would immediately start walking again. The development and progress of the disease was so insidious that her mother did not become particularly worried about it until long after its appearance, when the weakness of the legs had become sufficiently developed to interfere seriously with walking. At this time the child was 7 years old and could hardly walk across the room without falling. She never complained of pain, which probably was the main reason why so little attention was paid to her condition at its onset. Coincidental with the onset of this ailment she developed a marked ichthyosis of the lower extremities, which persisted throughout life and resisted all attempts at treatment. Also, her spine started to assume that peculiar exaggerated lateral curvature so characteristic in these patients.

Two years after the onset of illness, at the age of 7, she developed coarse twitching movements of the entire body, especially in the extremities, which would become exaggerated upon walking. A physician consulted concerning these singular movements made a diagnosis of chorea.

At about this same time her speech became slightly impaired. At times she could talk normally, but often her speech was drawling and difficult. Hearing and vision were not affected. Mentally the child seemed very bright. She attended school regularly and kept up with her class easily.

When the patient was 9 years old the first complete physical and neurological examination was performed, the details of which will be recorded later. At this time the mother had already noticed that the patient was retarded in her physical development. She had developed a marked atrophy of all the muscles below the knees and her legs were always cold and cyanotic. The weakness in the lower extremities increased and her gait became more and more difficult until, at the age of 11, six years after the onset of illness, she could no longer walk alone and was confined to a wheelchair. The disease had progressed so slowly that it was not until she was confined to a wheelchair that she discovered that the trunk muscles were too weak to support her body in the upright position without the aid of the upper limbs. Because of the progressive weakness in these trunk muscles and the increasing difficulty of supporting her body she became bedridden at the age of 14 years and was confined to her bed for the remaining 7 years of her life.

In September 1929, at the age of 18 years, she suddenly lost the use of her entire right upper extremity with the exception of the fingers. The left upper limb remained normal so that she was still able to feed herself and perform various tasks. However, even this extremity grew weaker, and by September 1932 she was unable to use any of her four limbs. This was her final condition on Dec. 22, 1932, when she succumbed to an attack of pneumonia.

Throughout her illness she was always happy and readily adjusted herself to her steadily progressing ailment. Mentally she remained bright to the very end. She read a great deal, her eyesight remaining fairly normal. Physically (exclusive of this neurological condition) she was well, although her appetite was poor and her development somewhat retarded. The menses were established at the age of 15 years and continued regularly until the time of death.

The father, aged 57 years, and the mother, aged 52 years, are both living and well. The parents do not know of any member of either branch of the family

who had an illness resembling in any respect the affliction of their daughters. The patient had one brother, aged 23 years, who is living and very robust. However, her sister, aged 18 years, has had a similar affliction since the age of 11 years. The history of the sister will be given later.

The most complete physical examination was performed in 1921 when the patient was 9 years old, four years after the onset of illness.

The examination revealed a fairly well developed and well nourished white female child. Her face had a peculiar expression because she kept her mouth open and the front teeth protruded over the lower lip. Ears, nose and throat were normal. There was a slight enlargement of the posterior cervical glands. The lungs were normal. The heart was slightly enlarged to the left, with a faint blowing systolic murmur at the apex. The abdomen was negative. The skin of the lower extremities was notably cooler than that of the rest of the body. The skin below the knees was thickened, dry, coarse and scaly. The spine was not tender but showed a marked scoliosis with a slight kyphosis. There was an extreme degree of hypotonia and muscular weakness, because of which the child sat and stood with the shoulders sagging and the head dropped forward.

The urine was normal. The red cell count was 4,630,000, hemoglobin 71 per cent, leukocytes 14,800 — polymorphonuclears 70 per cent, lymphocytes 30 per cent. The Wassermann and von Pirquet tests were negative. Creatinin was 0.6 mg., sugar 96 mg., and urea nitrogen 12.07 mg. per 100 cc. of blood.

Electrocardiographic examination revealed a right ventricular preponderance and diphasic second and third leads. Roentgen-ray examination of the skull showed no evidence of tumor or of increased intracranial pressure. Roentgen-ray examination of the heart and lungs was also negative.

Neurological Examination: The patient was well behaved and very coöperative in the examination. Her memory and intellect were normal and she manifested an active interest in the various findings. Her vision was unimpaired, pupils reacted to light and accommodation, eyegrounds were normal, and there was no nystagmus. At intervals her speech became slow and somewhat scanned, but no stammering or hesitancy was apparent. All voluntary movements could be executed but were manifestly ataxic. Position and muscle sense, as well as the superficial sensations, were absolutely normal in all their manifestations. The deep reflexes were not elicited — biceps, triceps, supinator, knee jerks and ankle jerks. The Babinski test was negative, but the Romberg test was markedly positive. The child had an ataxic gait, walking with the legs far apart, advancing slowly and with difficulty, dragging her feet at each step and tending to walk on the outer aspect of one sole and the medial aspect of the other. Her feet were small and deformed, and showed a pes cavus. A general hypotonia was very evident.

From the age of 9 years the patient had occasional examinations. The only change noted was a progressive advance of the motor paralysis.

CASE 2. Clinical History: The second case is that of L. C., 2 years younger than her sister A. C. The onset and progress of illness was very similar to that of her sister.

L. C. was born normally at full term and was in good health during infancy. She developed normally and walked at an early age. Quite intelligent, she attended school regularly and soon learned to read and write. At the age of 11 the first difficulty in walking appeared. (At this time A. C., her older sister, was 13 years old and had already completely lost the use of her lower limbs.)

This weakness in the lower extremities progressed slowly, her gait became more irregular and hesitating and she tired easily and often would have to sit down and rest. Nevertheless, she was able to walk alone easily. At the onset of her ailment her skin, like that of her sister, became coarse, scaly and thick. This ichthyosis was generalized, resisted all treatment and has persisted to the present time. She has never complained of pain.

The earliest defect in the movements of the hands developed about one year after the onset of illness, at the age of 12 years. This started as tremors or oscillations which showed themselves toward the end of a voluntary movement, being most marked when she attempted some coarse action such as reaching for an object and grasping it. In addition to this tremor of the hands, she soon developed involuntary jerky movements like those of chorea in the muscles of the extremities and neck, the head showing marked jerking movements when she attempted to speak.

At the age of 13 years she finished grade school work, well up in her class, but because of the progressive weakness in her lower limbs she was unable to continue her education. She continued to walk for two more years until the age of 15, but her gait was becoming more and more difficult and finally, in 1930, her legs had become so weak that she could no longer walk alone. However, she could move the legs well when lying on her back. At about this time the first difficulty of speech was noticed. Her speech would at times become jerky and somewhat scanned. Occasionally, in talking, there would be a sort of hesitancy, then a series of words would succeed one another rapidly in an explosive manner.

During the last two years she has failed to develop physically and has remained small in stature. There has developed a marked pes cavus and an exaggerated curvature of the spine. The strength and usefulness of her upper limbs has been retained to the present time. The menses were established at the age of 15 years and have been regular since. Vision has been normal, although at times she complains of a slight blurring and diplopia. The lower extremities feel cold and often turn blue. At no time has there been any sphincter disturbances, although recently the child has complained of some urinary frequency.

She was examined thoroughly for the first time in 1928, at the age of 14 years, three years after the onset of illness. At that time the muscular development of the face and upper part of the body was good. The lungs and heart were normal on physical examination. Blood pressure was 92/52. The abdominal examination was negative. The skin over the entire body was dry and scaly.

The urine was normal. The red blood count was 3,900,000, hemoglobin 74 per cent, white blood cells 3800 — polymorphonuclears 50 per cent, lymphocyte 48 per cent, monocytes 2 per cent. The Wassermann reaction was negative. The basal metabolic rate was +28.

Neurological Examination: There was no impairment of vision. The pupils were equal and regular, reacting to light and accommodation. The fundi showed no changes. Hearing was normal and speech was characterized by its monotony, with the exception of occasional explosive expressions. Swallowing was unimpaired. There was a marked ataxia of the upper extremities; the biceps, triceps, supinator, knee jerks, and Achilles' tendon reflexes were all absent; the joint sense in the hands and feet was, however, normal. There was a marked reduction in vibration sense over the lower extremities. The

abdominal and skin reflexes were all present and unimpaired, and the muscle tonus was markedly diminished over the entire body.

The last physical examination was performed by us in January, 1933. The findings will be given in more detail in order to make clear her present condition, and the progress of the disease since the previous examination five years ago.

The examination revealed a well nourished but imperfectly developed individual. As she sat in her wheelchair one noticed an occasional oscillation of the head from side to side, and purposeless jerking movements of the upper extremities. This was associated with a peculiar twitching and an exaggerated play of the facial muscles, especially while talking, which would spasmodically draw the lips upward into an empty grin. The skin over the entire body was coarse, thick and scaly. The mouth, teeth and tonsils were normal; no enlarged lymph nodes were palpable. The breasts were well developed.

The pulse rate was 75 and normal in volume. The lungs and heart were normal. The abdomen was negative. The genitalia appeared normal externally.

The sense of smell was unimpaired. Visual fields were normal, visual acuity good, ophthalmoscopic examination negative. There was a marked lateral nystagmus with the quick component in a direction opposite to the gaze. Her corneal reflexes were greatly reduced. Superficial sensation over the entire face was unaffected, and the motor fifth nerve also seemed normal. Her hearing appeared moderately reduced, but the test was somewhat unsatisfactory due to the lack of coöperation and the conditions under which the test was performed. Vestibular tests were not done. No difficulty in swallowing was apparent and both pulse and respiration were unaffected. She was able to protrude the tongue normally, with no impairment of its side to side motion.

There was a slight generalized reduction in the size of the extremities, which appeared quite small in comparison with the trunk. A moderate contracture was present in the lower limbs, the lower leg being flexed on the thigh. The great toes were raised up clawlike bilaterally, with an extension of the metacarpal-phalangeal joint and a flexion of the interphalangeal joints. The feet were small, deformed and somewhat shortened, with the sole of the foot hollowed, producing a typical pes cavus. There was an extreme flaccidity at the wrist and elbow joints, accompanied by a typical foot drop; however, the muscular strength of the arms and legs was well preserved.

None of the deep reflexes was elicited even with enforcement, but the abdominal reflexes were present and normal. She had a bilateral positive Babinski and no clonus. All voluntary movements of the arms and legs could be executed although manifestly ataxic; it was impossible for the patient to touch with the index finger the tip of her nose or her other index finger even with the eyes open. She could not walk, her legs apparently being unable to support the weight of her body, but when sitting or lying there was no impairment of the movements in the lower limbs.

The loss of position sense in both the upper and lower extremities was very striking since she could not distinguish, with her eyes closed, even gross changes in the direction of her fingers and toes. The integrity of deep tendon and muscle sense was not disturbed. No definite impairment in the superficial sensibility was elicited, although the results of this test were somewhat unsatisfactory because of the marked coarseness of the skin and the lack of coöperation on the part of the patient.

On examination of her back one noticed the marked scoliosis with the convexity to the right in the thoracic region, and to the left in the lumbar region.

Her speech was slow and somewhat scanned, but without hesitancy. There was no stammering, but often a hesitation before an answer, and then a series of words would succeed one another with an almost explosive rapidity.

DISCUSSION

The clinical history of our two cases furnishes a typical picture of the symptomatology of Friedreich's disease, which may be recapitulated as follows: A slowly progressive ataxia and weakness attacking two sisters, having its onset at the ages of 5 and 11 years respectively, beginning in the legs and extending gradually to the trunk and arms, but associated with almost no pain. There was also a slow, drawling scanned speech, choreiform unsteadiness, nystagmus, scoliosis, peculiar deformity of the feet (*pes cavus*), and abolition of the deep reflexes. To these we can add the negative findings of absence of pain, integrity of cutaneous sensation, integrity of sight, absence of genito-urinary troubles — the sphincter control being normal — and no luetic antecedents in the family. To complete the discussion of the clinical picture of Friedreich's ataxia a brief reference should be made to the literature since 1926 and to a few of the less frequent findings, such as occurred in our cases. About 25 cases that can be classified as Friedreich's ataxia have been reported since 1926.

In summarizing these cases the frequency of the most common findings was as follows: ataxia 100 per cent, loss or diminished deep reflexes 96 per cent, foot deformity 76 per cent, nystagmus 60 per cent, speech defect 60 per cent, spine deformity 56 per cent, positive Babinski 48 per cent, impaired deep sensation 36 per cent, impaired superficial sensation 12 per cent, muscle atrophy 16 per cent, and mental disturbances 4 per cent. Only one reference could be found in the literature to an accompanying ichthyosis which was so marked in both of our cases. Muscular atrophy was noticed only in our first case and as a rule is infrequent in this condition. The significance and interpretation of this atrophy will be brought out presently in the discussion of the pathological findings.

Because of the infrequency of detailed pathological studies in Friedreich's ataxia a careful report of our autopsy findings should be of interest.

PATHOLOGICAL REPORT

The autopsy showed an imperfectly developed and poorly nourished white female 143 cm. long, and weighing about 70 pounds. There was a marked scoliosis with the convexity to the right in the thoracic region, and to the left in the lumbar region. Both feet

TABLE I

Summary of Findings in 26 Cases of Friedreich's Ataxia

Author	Ataxia	Deformity of foot	Spinal deformity	Loss of deep reflexes	Nystagmus	Babinski's reflex	Speech defect	Impaired superficial sensation	Impaired deep sensations	Mental disturbances	Muscular atrophy
Beer, G.	+	+	+	?	..
"	+	+	+	+	+	..
"	+	+	..	+	+	+
Babonneix, L., & Schekter, L.	+	+	+	+	+
"	+	..	+	+	..	+
Alpers, B. J., and Waggoner, R. W.	+	+	+	+	+	+	+
"	+	+	+	+	+	+
"	+	+	..	+	+
"	+	+	..	+
Hiller, F.	+	+	+	+	+	+	+	..	+
"	+	+	+	+	+	+	+	..	+
"	+	+	+	+	+	+	+
"	+	+	+	+	+	+	+
Popow, N. A.	+	+	+	+
Rabinowitsch, V.	+	+	+	+	+	?	..
Euziere, J.	+	+	+	+	+	..	+
"	+	+	+	+	+	+	+	..	+
"	+	+	+	+	+	+	+
"	+	+	+	+	+	+	+	..	+
Orbán, A.	+	+	..	+	+	+	+
"	+	+	..	+	+	..	+
"	+	+	..	+	+
Salus, F.	+	+	+	+
Krebs, E., and Mollaret, P. ...	+	..	+	+	+	+	+
Pommé, B., <i>et al</i>	+	+	..	+	+	+	..	+

showed a pes cavus. Each extremity measured 73 cm. from the anterior superior spine to the external malleolus. The left calf was 22 cm. in circumference, the right 20 cm. The left middle thigh measured 29 cm., the right 27.5 cm.; the left middle arm 18.5 cm., the right 17.5 cm. The muscles of the abdomen were fairly well developed.

The peritoneal cavity was normal. The diaphragm was at the level of the fourth interspace on the right, and the fifth rib on the left. The pleural cavities and pericardial sac were normal. The heart, root of the aorta and coronary arteries were normal.

The right lung weighed 250 gm., and its lower and middle lobes contained patchy areas of bronchopneumonia. The left lung weighed 150 gm. and was normal both externally and on section. Neither lung contained carbon pigmentation, bearing strong evidence of the indoor life of the patient.

The spleen, liver, kidneys and pancreas were all normal.

The muscles generally appeared fairly normal, with the exception of those in the lower extremities, which were largely replaced by adipose tissue. Microscopic examination of the atrophic muscles revealed a widespread but patchy degeneration of muscle with replacement by adipose tissue. In the persistent muscle bundles there were scattered groups of atrophic fibers in which the sarcoplasm had partially or completely disappeared. The sarcolemma shrinks to fit the reduced sarcoplasm but the muscle nuclei persist.

A striking change was found in the small intramuscular nerves. Almost all of these nerves showed a marked proliferation of the Schwann cells with replacement of nerve fibers, giving the nerve a sclerotic appearance. The structure of the nerves will be described more fully in a subsequent paragraph.

The entire spinal cord and many of the peripheral nerves were removed for further study. On gross examination the entire spinal cord was unusually small, appearing about one-half the normal size. The dura mater and the pia arachnoid were normal.

Sections were taken from various levels of the cord and stained in the following ways: hematoxylin-eosin, Weigert's myelin sheath stain, iron hematoxylin-Van Gieson, Nissl's method, azocarmine and Bielschowsky's stain.

The cervical cord presented the most marked changes. With the Weigert stain almost complete demyelination was noted in the columns of Goll and Burdach, the lateral and ventral corticospinal tracts (crossed and direct pyramidal), and in the dorsal spinocerebellar tract (Fig. 1). The gray matter also presented a few alterations. The anterior horn cells were diminished in number, although none of them showed chromatolysis. No cells were visible in the posterior horns, which appeared somewhat vacuolated and contained a

number of dilated blood vessels. With the hematoxylin-eosin and the iron-hematoxylin-Van Gieson stains the cervical cord showed a moderate gliosis in the middle zone of the posterior and lateral columns.

The anterior columns were intact. The anterior rootlets were essentially normal. The myelin sheaths of the posterior rootlets were completely degenerated, while their axis cylinders were, for the most part, swollen or entirely absent. There was a marked Schwannian proliferation replacing many of the degenerated rootlet fibers.

No signs of inflammation were apparent. There was no cellular infiltration into the cord or around the blood vessels. The blood vessels themselves appeared normal.

In the midthoracic cord the changes were the same as in the cervical, except that demyelination was not so pronounced in the pyramidal tracts. The anterior horn cells appeared normal both in number and in structure. An occasional shrunken nerve cell without a visible nucleus was found in the posterior horn. The cells of Clark's column were diminished in number, only a few appearing in the section. They were shrunken and irregular, the Nissl bodies were clumped, the nuclei, when present, were eccentrically placed (Fig. 3). There was only a minimal amount of gliosis present in the posterior and lateral columns.

The posterior rootlets were more involved than in the higher cord levels. They showed a marked destruction of both the myelin sheath and the axis cylinder, with a proliferation of the Schwann cells. In spite of the almost complete destruction of many posterior rootlets, a few areas were found with intact fibers. These persistent fibers probably accounted for the fact that some of the patient's superficial and deep sensations were intact. The anterior rootlets were unaffected.

The arteries around the cord were normal.

In the lumbar cord the posterior columns and the dorsal spinocerebellar tracts were still completely degenerated (Fig. 2). The crossed pyramidal tract, due to its actual decrease in size, appeared less prominent at this level. There was no sclerosis present. The anterior horn cells and the anterior rootlets were unaffected. Only a few intact fibers were found in the posterior rootlets (Fig. 2).

Some striking changes were found microscopically in the peripheral nerves. Only those of the extremities were removed for study

and, since the alterations in all were essentially the same, a detailed description of the changes in the sciatic nerve alone will be given.

With the Weigert's stain there appeared a patchy degeneration throughout the proximal part of the nerve (Fig. 4). In the distal portion, besides a similar patchy demyelination, many of the nerve fibers showed a marked uniform swelling of their myelin sheaths. The axis cylinders were, as a rule, well preserved but in some regions, especially in sections taken from the distal segments of the nerve, they were somewhat swollen and often entirely absent (Fig. 4).

Another conspicuous feature in all the sections studied was the overgrowth of Schwann cells (Fig. 4). This proliferation was irregular and the manner of its distribution varied, even within a single section. In some funiculi this cellular overgrowth was limited to single or multiple well defined areas (Fig. 4), while in others it occurred as a diffuse proliferation which only partly replaced the nerve elements. In some areas, however, this Schwann cell proliferation was so marked that entire nerve bundles were replaced by large groups of cells and dense masses of fibrous tissue.

In the pathological reports found in the literature and well reviewed by Barrett, the most common lesions were a diminution in the size of the spinal cord, sclerosis of the posterior columns and degeneration of Clark's cells. Often a degeneration of the dorsal spinocerebellar tract occurred and less frequently a partial involvement of the crossed pyramidal tracts and posterior rootlets. Only rarely was there any mention of changes in the peripheral nerves. This may be due either to the absence of lesions or to the fact that the nerves were not examined at postmortem. Since Barrett's publication there have apparently been no autopsy reports on cases of Friedreich's ataxia, although there have been numerous clinical reports.

Friedreich himself called this complex hereditary ataxia. However, since his time it has generally been stated that Friedreich's ataxia is a familial but not a hereditary disease, appearing in families, usually involving a few members, but in many instances affecting a single individual. Indeed, from our histories it would seem that this was true, for there was no suggestion of a hereditary tendency in our two cases. But Friedreich's ataxia has been detected in several generations of a family as reported by Orbán, and others. These cases, although few in number, at least suggest a tendency to direct in-

heritance in this condition. Alpers and Waggoner have probably truly stated the situation when they write that Friedreich's ataxia is a "hereditary disease which often seems to appear sporadically in families, involving either a single or many members in the group."

SUMMARY

1. Two typical cases of Friedreich's ataxia are presented, appearing in two sisters and presenting almost identical symptomatology.
2. A detailed postmortem report is given of one of the cases, with special emphasis on the histological changes in the peripheral nerves.
3. A brief summary is given of the literature since 1926.

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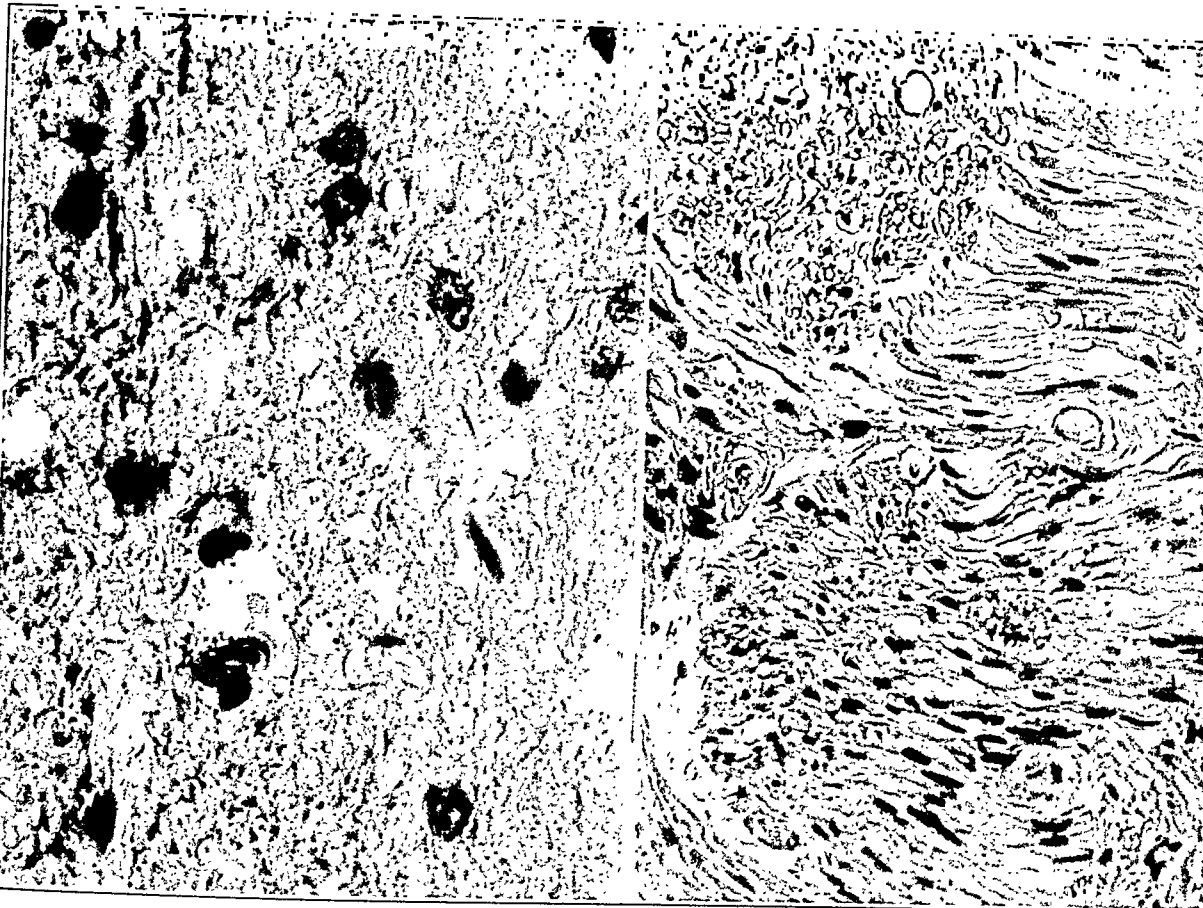
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DESCRIPTION OF PLATE

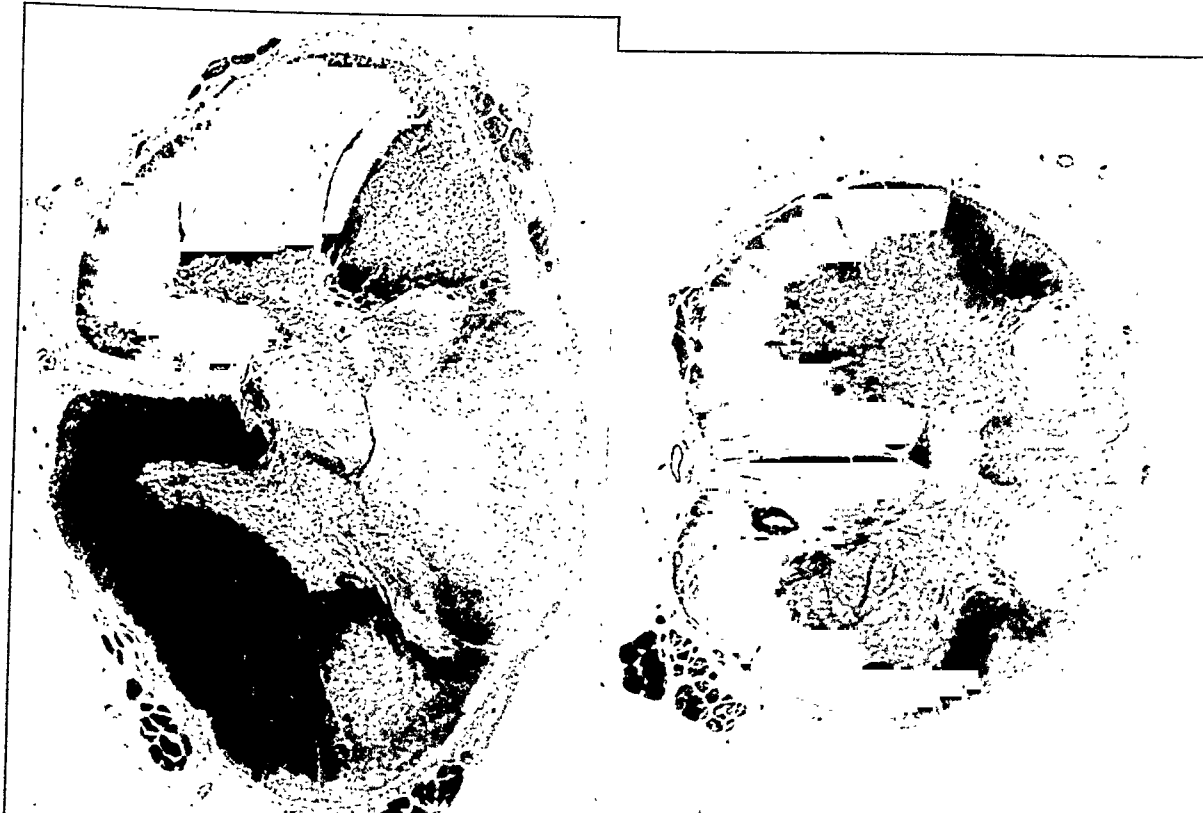
PLATE 50

- FIG. 1. Section through the cervical cord. Weigert's myelin sheath stain. Note the destruction of the posterior and lateral columns.
- FIG. 2. Section through the lumbar cord. Weigert's myelin sheath stain. There is a degeneration of the posterior columns and of the dorsal spinocerebellar tracts. Note the marked destruction of the posterior rootlets as compared to the anterior.
- FIG. 3. Section through Clark's column in the midthoracic cord. There is a marked shrinking and irregularity of the cells as well as a reduction in their number.
- FIG. 4. Section of the sciatic nerve. Iron hematoxylin-Van Gieson stain. Note the striking overgrowth of Schwann cells replacing the nerve fibers.



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PRIMARY NEUROGENIC SARCOMA OF THE BLADDER IN AN INFANT 1 MONTH OF AGE *

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The patient, a white female, 1 month of age, was admitted to the New Haven Hospital on July 7, 1932, gravely ill and in a condition of extreme malnutrition. The child was born in the hospital on June 7, 1932, after a protracted and difficult labor in which the mother's cervix was dilated by means of a Voorhees' bag and the delivery effected by breech extraction 24 hours after the onset of labor. The child cried spontaneously but because of cyanosis and partial pulmonary atelectasis carbon dioxide and oxygen were administered at intervals for 2 days. The physical examination at birth revealed a normal infant weighing 2810 gm. When feedings were started the child experienced some vomiting and diarrhea, but otherwise progressed favorably during the 10 days she remained in the hospital. Because of the mother's lack of intelligence little could be learned of the infant's condition prior to its return on July 7th. At this time the mother brought the child to the hospital after friends had called attention to its poorly nourished state. Physical examination revealed an extremely cachectic and acutely and gravely ill infant who weighed but 2330 gm. The temperature was unobtainable since it was below 34° C. The abdomen was scaphoid and soft. A firm, symmetrical, non-tender mass, which arose from the pelvis and extended halfway to the umbilicus in the midline was readily palpable. It was thought to be the distended bladder.

Immediately upon arrival at the hospital the child was given supportive treatment to combat the marked dehydration, but death occurred 18 hours after admission.

POSTMORTEM FINDINGS

The body was that of a well developed but poorly nourished female infant weighing 2350 gm. The skin over the body was thin, loose and

* Received for publication August 16, 1933.

inelastic but everywhere intact. There were no nodules, zones of pigmentation or nevi present in the skin.

The only significant changes in the viscera were confined to the genito-urinary tract (Fig. 1). When the peritoneal cavity was opened a firm, pale, ovoid mass, which extended 4 cm. above the symphysis pubis in the midline, was seen. This was the greatly enlarged and thickened urinary bladder, which measured 4 by 2 by 2 cm., and presented a uniformly smooth, even, external surface.

When the bladder was opened its wall was found to be thickened by dense masses of pale, translucent tissue that diffusely infiltrated the submucosa and extended haphazardly into the muscularis. The tumor masses appeared to be made up of pale yellow fibrils arranged in a whorl-like fashion. The wall of the bladder was irregularly thickened; the most extensively involved portions reached a diameter of 1.5 cm., while the less involved measured 0.5 cm. The mucosa of the bladder was everywhere intact and pale in appearance. In the thicker portions the tumor masses bulged into the lumen of the bladder and rendered its inner surface irregular in contour and greatly decreased its capacity. The greatest involvement was in the region of the trigone where the tumor masses impinged upon the ureteral orifices and produced a bilateral hydro-ureter and hydro-nephrosis. In this region the tumor had extended through the wall of the bladder to invade the vesicovaginal connective tissue and the outer layers of the cervical uterine wall. This extension did not alter the contour of the uterus or vagina, nor did it encroach upon the lumen of the urethra. The rectum was entirely free from invasion.

MICROSCOPIC EXAMINATION

The epithelium of the bladder is intact, but in many places it is raised in irregular fashion by masses of neoplastic tissue that infiltrate the submucosa extensively. The tumor tissue invades the muscularis and in several zones has reached the external surface of the bladder. The neoplasm is principally composed of bundles of fine pink-staining fibrils, throughout which are interspersed elongated fusiform cells with blue-stained oval nuclei. Clustered about these bundles are masses of cells with scant cytoplasm and large blue vesicular nuclei that contain a distinct chromatin network. These cells vary in size and shape. Many are round, others pyriform, and

frequently fibrillary processes appear to extend from them into the bundles. Occasionally small groups of these cells tend to simulate an acinar-like arrangement. Rarely cells are seen in a state of karyokinetic division. Distributed throughout the tumor are small numbers of large pyramidal cells with a dense homogeneous violet cytoplasm and large vesicular nuclei, in each of which a distinct central nucleolus is visible. Frequently these cells are multinucleated. In other portions the tumor is composed of smaller bundles of closely packed fusiform cells which present a wavy appearance. Usually associated with these are numerous ball-like structures made up of a central pink-stained fibrillar mass surrounded by one or more layers of spindle-shaped cells with pale blue oval nuclei. Frequently the fibrillary processes appear to extend from these cells into the central core.

In Mallory aniline blue preparations a distinct network of fine collagenous fibers permeates the tumor tissue and surrounds the various components which stain bright pink to violet.

Pal-Weigert myelin sheath stains demonstrate occasional myelinated nerve fibrils scattered throughout the neoplasm.

DISCUSSION

The diagnosis of neurogenic sarcoma, in this case, is based on the presence of fibrillary structures associated with clusters of primitive cells and fragments of myelinated nerve fibrils which invade the wall of the bladder extensively. The masses of undifferentiated cells intermingled in bundles of fibrils, together with the large pyramidal cells and the ball-like structures, are features of the tumor that strikingly resemble the neoplasms described by Wright,¹ Pick,² Wolbach and Morse³ and others, under the name of neurocytoma or neuroblastoma sympathicum. The presence of myelinated nerve fibrils, however, rather suggests the assumption that this tumor may not be derived from sympathetic nervous tissue elements. Furthermore, the scant amount of myelin present in the neoplasm may be due either to the embryonal nature of the tissue or to the fact that at the age of the patient myelin may not be formed. A source of origin of the neoplasm is readily accounted for in that the wall of the bladder has a rich plexus of myelinated nerve fibers derived from the lower sacral segments of the spinal cord. Although malignant tumors

arising from nervous tissue elements are known to develop in the adrenals and the retroperitoneal tissue of infants, the occurrence of such tumors primary in the wall of the urinary bladder has not been reported.

The rarity of primary neoplasms of the bladder in infancy is clearly brought out in Deming's review of the subject,⁴ where only four instances are recorded in children under 1 year of age. Three of these were fibrosarcomas, the fourth a polyp. The only types of bladder neoplasms of nervous tissue origin so far reported are neurofibromas⁵ associated with generalized von Recklinghausen's disease.

SUMMARY

A case of neurogenic sarcoma arising in the wall of the bladder of an infant 1 month old is reported. This tumor resembles the group of neoplasms classed as neurocytomas, or neuroblastoma sympathicum.

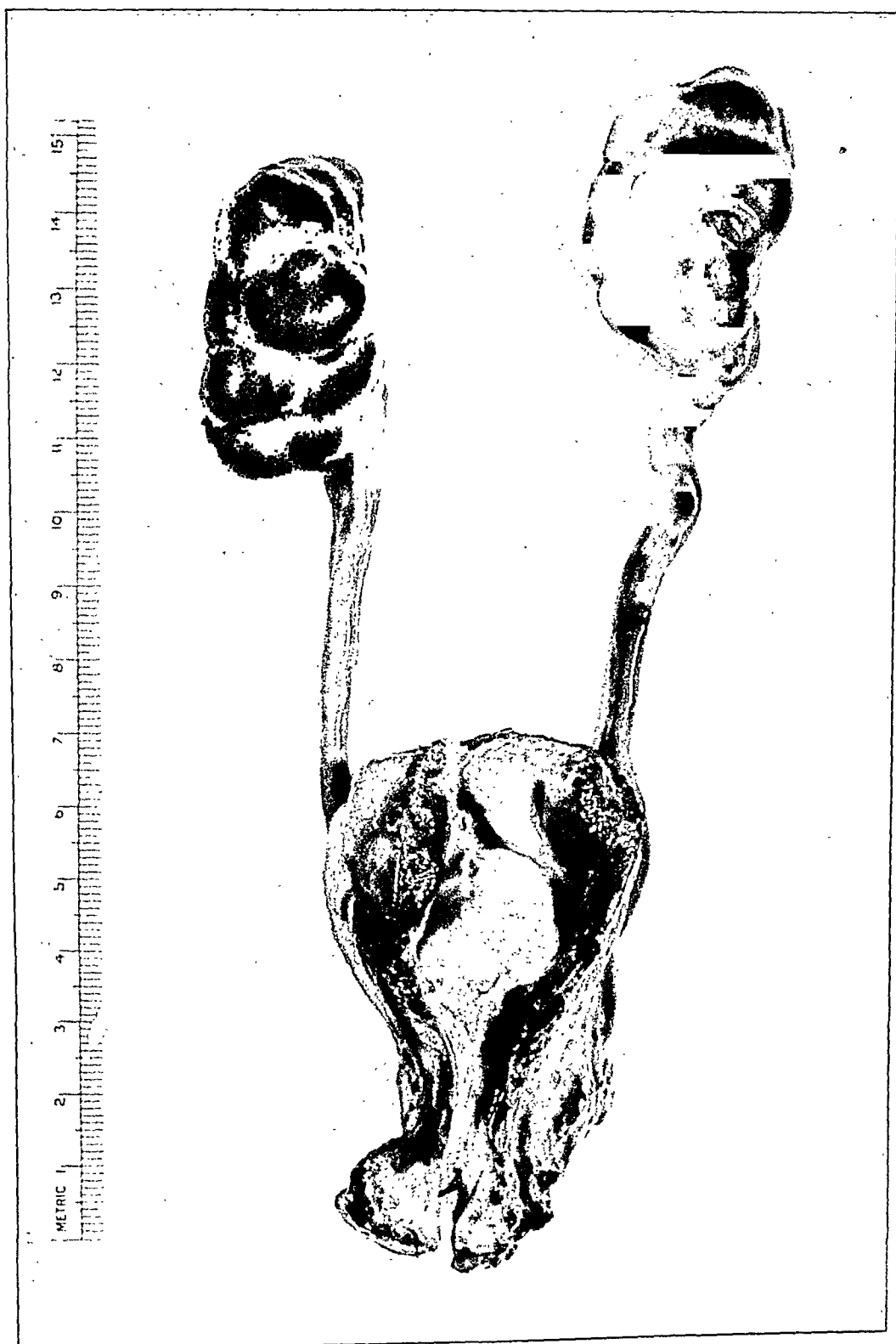
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DESCRIPTION OF PLATES

PLATE 51

FIG. 1. Bladder opened anteriorly to show main tumor mass bulging into trigone, with thickening of wall and associated bilateral hydro-ureter.

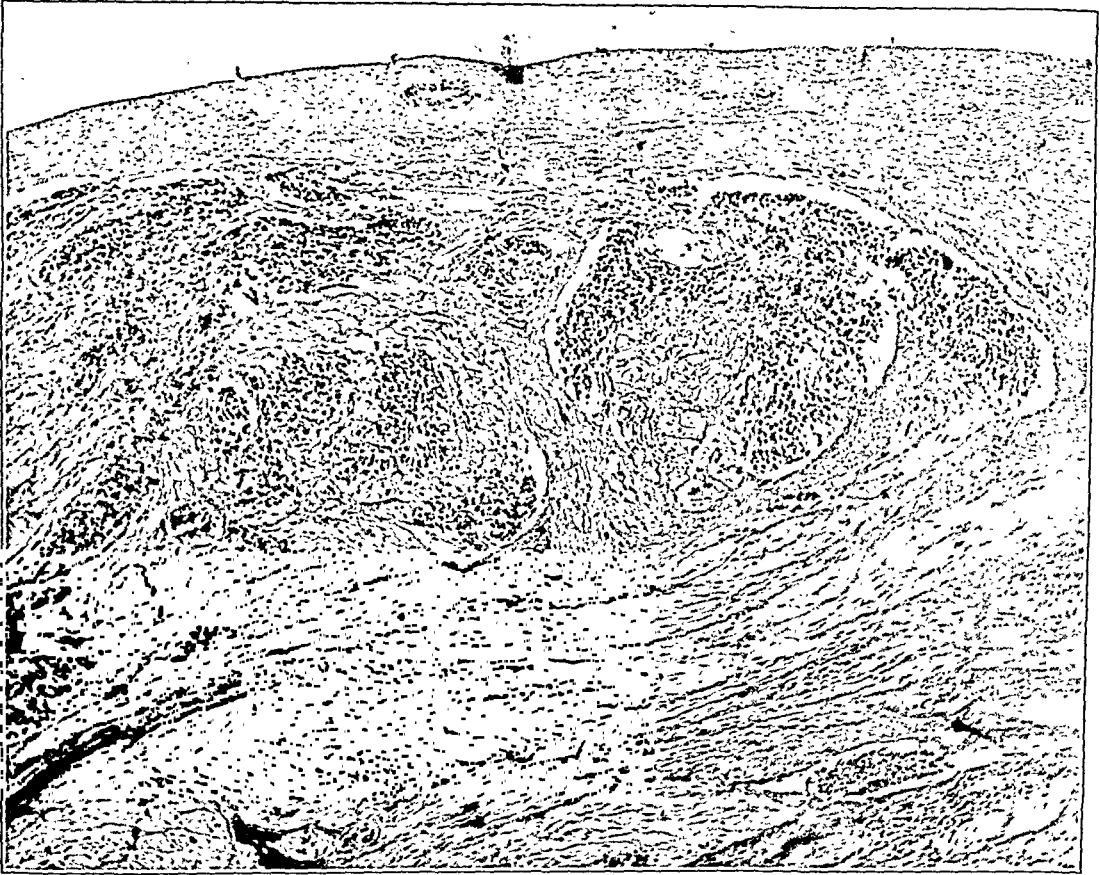


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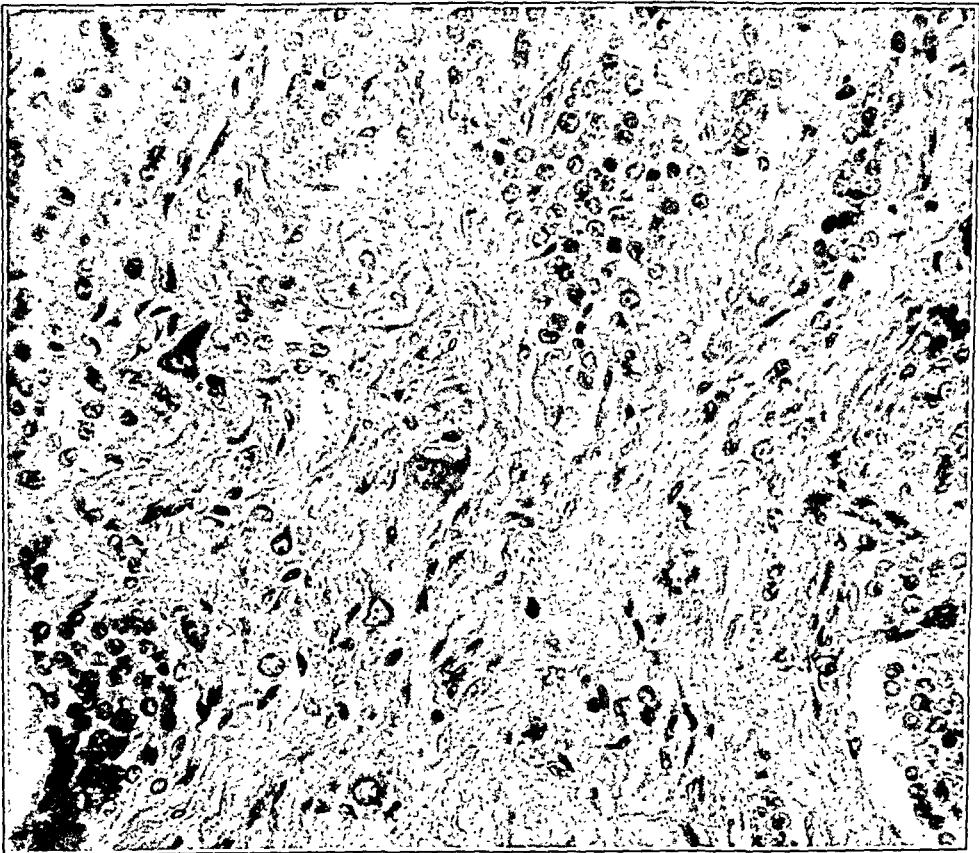
PLATE 52

FIG. 2. Tumor in submucosa of bladder wall showing bundles of fibrils and clusters of small undifferentiated cells. Hematoxylin and eosin stain. $\times 80$.

FIG. 3. Large pyramidal cells and masses of small undifferentiated cells intermingled with fibrils. Hematoxylin and eosin stain. $\times 225$.



2



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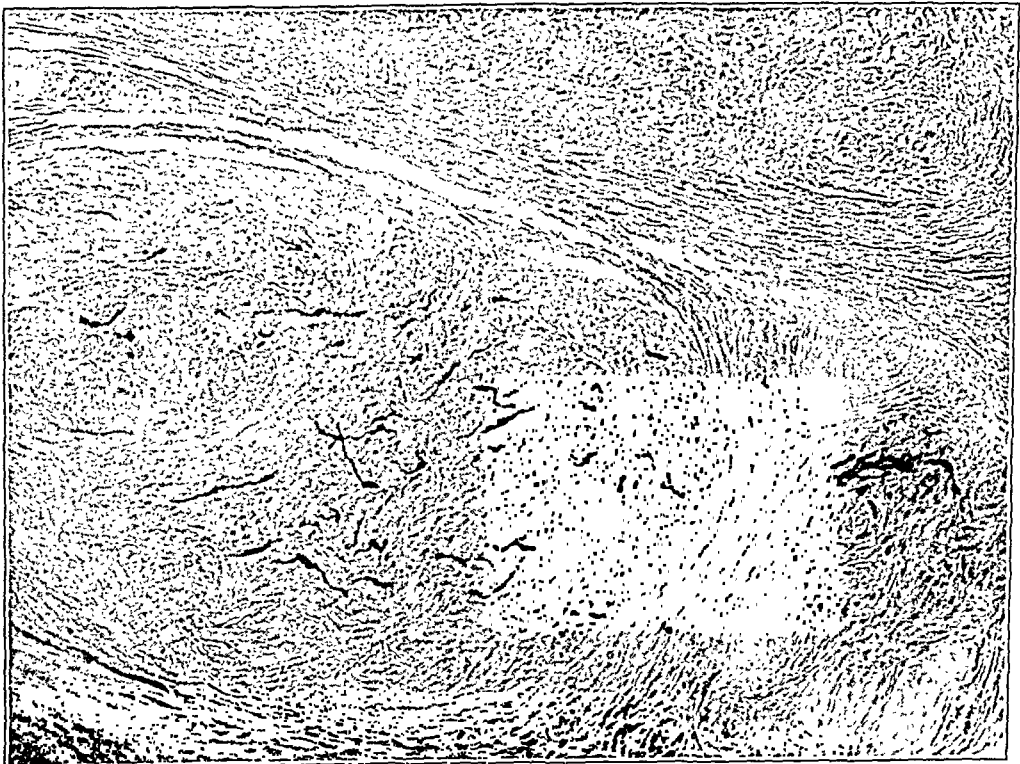
PLATE 53

FIG. 4. Ball-like structures with surrounding fibrillar bundles. Hematoxylin and eosin stain. $\times 125$.

FIG. 5. Myelinated nerve fibrils in tumor mass. Pal-Weigert myelin sheath stain. $\times 150$.



4



5

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THROMBOSIS AND PULMONARY EMBOLISM *

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Observations made upon a comprehensive series of autopsy cases from the services of a general hospital impress one with the fact that pulmonary embolism is a much commoner occurrence than it is ordinarily considered to be. Usually regarded as a postoperative complication of relatively rare occurrence it proves, on the contrary, to be a common autopsy finding, more often associated with medical than with surgical cases. The frequency with which it may be uncovered at autopsy is surprisingly high, as many pathologists have already pointed out (Lubarsch,¹ Fahr,² Henderson,³ Singer,⁴ Wertheimer,⁵ Dietrich,⁶ Benda,⁷ Ceelen,⁸ Axhausen,⁹ Putnoky and Farkas¹⁰). On our own service, under the direction of Prof. Oskar Klotz, we have demonstrated pulmonary emboli in about 10 per cent of our routine autopsies and our observations have prompted this communication in the hope of drawing further attention to the seriousness of the problem, and in order to indicate some factors that seem of etiological significance.

The term *pulmonary embolus* is used here in a restricted sense, referring only to a wandering ante mortem blood clot of a size appreciable to the naked eye, lodged within the pulmonary arterial system or the right heart. It is impossible, as Dietrich⁶ observes, to make a sharp distinction between infected and non-infected emboli. One can, however, make a rough but serviceable distinction on other than bacteriological examination between bland and septic emboli, and it is our purpose here to consider only those cases in which the emboli seemed of a bland character and did not arise from suppurative foci.

The frequency with which pulmonary emboli are found at autopsy depends to a great extent upon the vigilance and technique of the observer. There is, perhaps, no other autopsy finding which is more readily passed over. Unless the personnel of an autopsy service has

* Received for publication August 10, 1933.

TABLE I

Autopsy Cases of Chronic, Congestive Heart Failure with Pulmonary Embolism (Group 1)

Autopsy number	Age	Sex	Type of heart disease	Emboli of size to cause death	Site of thrombosis	Clinical evidence of thrombosis	Infarcts of lung
208/31	yr. 48	M	Hypertensive	+	Prostatic, leg veins and right heart		+
346	51	F	Rheumatic	+	Brachial and pampiniform	Swelling of arm	+
369	18	F	Rheumatic	+	Leg veins and right heart		+
371	45	M	Rheumatic	+	Leg veins	Varicose veins	+
414	70	M	Coronary	+	Prostatic and iliac veins		+
12/32	70	F	Coronary		Right heart		+
13	63	M	Coronary	+	Leg veins	Varicose veins	+
45	41	M	Rheumatic		Not found		+
66	36	F	Rheumatic	+	Leg veins and right heart	Edema of leg	+
107	46	F	Syphilitic aortitis	+	Right heart		+
143	71	M	Coronary	+	Right heart		+
241	51	M	Pericarditis	+	Right heart		+
254	23	M	Rheumatic	+	Right heart		+
316	28	M	Rheumatic	+	Not found		+
342	64	M	Coronary		Right heart		+
378	60	M	Coronary		Not found		+
400	63	M	Coronary		Prostatic veins		+
21/33	43	F	Hypertensive	+	Leg veins		+
35	50	M	Coronary	+	Femoral and ovarian veins		+
36	68	M	Coronary		Leg veins		+
48	60	M	Coronary		Jugular		+
111	77	F	Rheumatic	+	Not found		-
121	69	F	Coronary	+	Leg veins	Edema of leg	+
131	54	F	Rheumatic	+	Common iliac		+
130	68	M	Coronary		Leg and iliac		+
					Prostatic		+

TABLE II

Autopsy Cases of Pulmonary Embolism in Cachectic or Debilitated Individuals with Various Fatal Diseases (Group 2)

Autopsy number	Age	Sex	Medical or surgical	Disease	Emboli of size to cause death	Site of thrombosis	Clinical evidence of thrombosis	Infarcts of lung	Evidence of heart disease
329/31	ys. 56	F	M	Liver cirrhosis	-	Not found		-	Acute verrucose, chronic sclerotic mitral endocarditis
368	24	M	S	Amyloidosis	-	Iliac vein		+	
48/32	90	M	S	Pyelonephritis with uremia	-	Iliac vein		-	Dilatation of right heart
130	59	F	M	Pyelonephritis with uremia	-	Femoral	Edema of leg	-	Dilatation of heart
158	69	M	S	Carcinoma of stomach	-	Leg veins		-	Brown atrophy of heart
191	49	M	M	Sarcomatosis	+	Femoral		-	Dilatation of right heart
291	49	M	M	Carcinoma of lung	+	Superior vena cava, pudendal		-	
338	53	M	M	Carcinoma of lung	+	Prostatic		-	Hypertrophy and dilatation of right ventricle
412	68	M	S	Carcinoma of stomach	-	Leg, pelvic		+	Brown atrophy and fibrosis of heart, passive congestion of liver
424	79	M	M	Brain tumor	-	Femoral	Edema of leg	-	Fatty infiltration of heart
16/33	68	F	M	Undetermined	+	Leg veins		-	Fatty infiltration of heart
19	75	M	S	Injury	-	Prostatic		-	Fibrosis of heart, diffuse dilatation of aorta
49	49	F	M	Diabetic gangrene	-	Common iliac	Gangrene of leg	+	Rheumatic heart disease
91	41	M	M	Sarcomatosis	+	Inferior vena cava		-	(Pressure on inferior vena cava)
124	40	F	M	Carcinomatosis	-	Iliac and inferior vena cava		-	Pericarditis
132	60	F	M	Sarcoma	+	Leg veins		-	(Pressure on inferior vena cava)

its attention especially directed to the problem there are bound to be many instances of pulmonary embolism that are not detected. Statistical studies of the autopsy incidence of pulmonary embolism, such as that undertaken by Rosenthal,¹¹ are therefore open to criticism on the grounds that figures from some clinics are much more carefully compiled than others. In another article¹² we have described at some length the special care that must be taken in order not to miss thromboses and emboli at postmortem examinations. Much has been written in recent years purporting to show an increase in the incidence of pulmonary embolism since the World War (see the reviews of Ceelen,⁸ Dietrich,⁶ Rosenthal¹¹), but that such an increase may not be due to more careful autopsy examinations is open to question. To distinguish between embolus and autochthonous thrombus in the pulmonary arteries often presents difficulties, as has been indicated elsewhere.¹² Doubtful cases are, however, excluded from our series.

Our observations were made upon a series of 567 complete autopsies on adults during an eighteen month period ending April, 1933. In the series were 56 cases of pulmonary embolism. Thirty-seven of these showed emboli of sufficient bulk to occlude two thirds or more of the pulmonary circulation, and were therefore regarded as the immediate cause of death (Dietrich⁶). In the other 19 cases the emboli were small and of a non-fatal character. Medical cases predominated over surgical in the ratio of 40 to 16. In all but one or two cases there were multiple emboli, illustrating a fact, which we have previously emphasized,¹² that pulmonary embolism is, as a rule, not a single but a recurrent event, with repeated migrations of blood clot over a period of hours or days, leading up to a fatal termination in the event of two-thirds or more of the pulmonary circulation becoming occluded. Ljungdahl¹³ described pulmonary embolism as being sometimes of a chronic nature.

A striking feature was the infrequency with which venous thrombosis was recognizable clinically. Probably as high as 60 per cent of the emboli arose from clotted leg veins (see Tables I, II and III), vessels relatively easy to examine and easy enough of detection when they are painful, yet the majority came to autopsy without recognition of the primary thrombosis during life. Three presented clinical thrombophlebitis with edema of one leg, and tender, indurated veins. Two more had gangrene of the lower extremities (due to arterial

TABLE III

Cases of Sudden Death from Massive Pulmonary Embolism in Convalescent Individuals (Group 3)

Autopsy number	Age	Sex	Medical or surgical	Disease preceding embolism	Days of illness	Site of thrombosis	Clinical evidence of thrombosis	Infarcts of lung	Evidence of heart disease
361/31	yrs. 50	F	S	Varicose veins injected	21	Leg veins	Varicose veins	-	Fatty infiltration
363	70	M	S	Bladder lithotomy	5	Not found		+	Coronary disease
400	76	F	S	Fractured femur	21	Leg and iliac veins		+	Fibrillation, fatty infiltration
87/32	58	M	M	Pneumonia	22	Femoral		?	
95	59	F	M	Pernicious anemia (re-mission)		Femoral		-	Fatty degeneration
117	39	M	M	Pneumonia	16	Not found		-	
170	58	M	S	Appendectomy	Few	Inferior vena cava		+	?
176	67	M	S	Stomach resection	3	Not found		+	?
229	44	M	M	Infective arthritis	Several	Leg veins		-	
284	67	F	S	Fractured femur	10	Not found		-	Fibrillation
386	64	M	S	Appendectomy	7	Leg veins	Varicose veins	-	
408	62	F	S	Ovarectomy	5	Leg veins		-	
23/33	64	M	S	Prostatectomy	2	Leg veins	Varicose veins	+	Coronary disease
110	44	M	S	Gastric resection	12	Internal iliac		+	
145	65	F	S	Fractured femur	8	Leg and iliac veins		-	Fatty infiltration

occlusion) and presented extensive venous thrombosis. Five additional cases were known to have varicose veins of the legs, but in no case were the varices tender nor did they show signs of active thrombophlebitis.

Of the 5 cases presenting evidence of active thrombophlebitis of the legs only 1 suffered a fatal embolism; the other 4 had but small emboli. As a general rule, the greater the local reaction accompanying a venous thrombosis the less the likelihood of large emboli breaking off. If, in and around a vein, there is an inflammatory reaction of sufficient intensity to create local manifestations the thrombus is likely to be securely attached to the vessel wall and only small masses are capable of dislodgement.

In the majority the thrombus was of the so-called spontaneous variety, developing without local signs. Four times out of five the resultant emboli were of a fatal character. Often enough it was impossible to determine the site of the parent thrombus, sometimes because it was not permitted to make a free dissection of the veins, sometimes because the entire thrombus had evidently migrated from its point of origin leaving no trace. In 8 cases the emboli closely resembled casts of femoral or saphenous veins but these vessels were found to be empty and apparently unaltered. Sixteen cases of spontaneous thrombosis with embolism showed remnants of thrombus in veins of the thigh. Six times the remnant was lying free in the vessel, ten times it was adherent to the intima and extended peripheralwards in one or more small tributaries. Similar adherent remnants were found three times in the internal iliac vein. Microscopic examination of these adherent thrombi showed no significant inflammatory reaction in the vein wall. The clot was made up of a compact platelet coagulum and was blended with the intima by a bland hyaline substance.

For purposes of further analysis our 56 cases fall into three fairly well defined clinical groups. The first and largest is the "heart failure" group in which 25 cases are found (Table I). All of these individuals were in a state of chronic invalidism from congestive myocardial failure, actual or impending. Twelve had coronary disease, 9 rheumatic heart disease, 2 hypertensive heart failure, 1 syphilitic aortitis and 1 pericarditis. Their ages varied from 18 to 77 years, with an average of 53. In only 5 of this group was there clinical evidence of venous thrombosis. The sites of thrombus

formation, as determined at autopsy, were as follows: leg veins 8, leg veins and right heart 4, right heart alone 5, prostatic veins 2, brachial and jugular veins each 1, not determined 4. In 15 cases the emboli were of a fatal character. All of this group except 2 showed infarcts of the lungs. In many instances the infarcts were of varying ages, indicating repeated emboli.

The second group comprises 16 cases, all of which were cachectic or debilitated with some incurable disease (Table II). Nine had inoperable malignant tumors. In 6 the emboli were large enough to cause death. The sites of thrombosis were: leg veins 6, femoral alone 3, iliacs alone 2, inferior vena cava 1, pelvic veins (prostatic, internal iliac, ovarian) 3. Three had infarcts of the lungs, 11 more showed pathological changes in the heart, so that 14 may be regarded as cases with impaired cardiac function. Two additional cases in the group, A-91-33 and A-132-33, had the inferior vena cava compressed by an intra-abdominal tumor. The emboli in each case arose from thrombi formed below the point of compression.

The third group comprises cases of sudden death from massive pulmonary emboli occurring in convalescent individuals who otherwise had a normal expectancy of life. Major interest attaches to these cases because of their dramatic clinical aspect. Fifteen cases fell into this group (Table III), 11 of them surgical and 4 medical. Six of the surgical cases followed laparatomies, 3 fractured femurs, 1 injection of varicose veins and 1 transurethral prostatectomy. The medical cases were made up as follows: 2 convalescent from lobar pneumonia, 1 recovering from infective arthritis and 1 pernicious anemia case in a phase of remission. The age variation in this group was from 39 to 76, with an average of 59. Three cases were known to have varicose veins of the legs, but in the other 12 there was no clinical evidence of venous thrombosis. Five of the 15 cases were diagnosed as embolism clinically. At autopsy venous thromboses were found in the following sites: leg veins 6, femoral alone 2, inferior vena cava 1, internal iliac 1. In 5 cases no thromboses were found. Six had infarcts of the lungs, a finding of twofold significance indicative firstly of repeated emboli occurring hours or days before the fatal issue, and secondly, affording evidence of impaired myocardial function to be considered more fully presently. In an additional 4 cases there was both clinical and pathological evidence of a damaged myocardium, 2 showing fatty infiltration, 1 fatty degeneration and

1 coronary disease. Thus, of 15 cases of sudden death from massive pulmonary emboli, 10 presented evidence of an impaired heart action.

Thirty-two out of 56 cases of pulmonary embolism had hemorrhagic, bland infarcts of the lungs. As has been shown by Karsner and Ash,¹⁴ occlusion of a pulmonary artery is not productive of infarct unless the venous return from the lungs is impeded. Such an impediment may be brought about by compression or thrombosis of the pulmonary veins or by stenosis or insufficiency of the mitral valve. In our cases we were able to exclude, with reasonable certainty, obstruction to the pulmonary veins and were therefore obliged to account for the infarction on the grounds of inadequacy of the mitral valve. In 8 cases there was definite mitral stenosis and macroscopic evidence of chronic passive congestion (brown induration), but in the other 24 there was no demonstrable lesion of the mitral valve, save, in some instances, a moderate dilatation of the valve ring. A mitral systolic murmur had been noted clinically in a number of these cases.

The high correlation between heart failure and pulmonary embolism led to a consideration of all the cases of congestive heart failure in the series of 567 autopsies. There were 83 such cases. As we have seen, 25 of these had pulmonary emboli. An additional 11 cases showed thrombi capable of giving rise to pulmonary emboli; 7 had thromboses of leg or pelvic veins, and 4 mural thrombus of right auricle. Thus, out of 83 cases of congestive heart failure 36 had thromboses on the venous side of the circulation. In 20 of these cases the thrombosis occurred in leg or pelvic veins, and was discovered only at autopsy; there were no local manifestations during life and the microscopic examination of representative examples showed nothing more than a few lymphocytes as evidence of inflammatory reaction in the wall of the veins. In all probability there was a higher incidence of venous thrombosis in this group of cases than could be uncovered at autopsy. It is impossible to examine all the veins in the course of a routine autopsy and consequently it is impossible to determine the incidence of venous thrombosis accurately.

To summarize, 25 of the 56 cases were invalidated with myocardial insufficiency, and 24 more presented evidence of a disordered heart with either infarcts of the lungs, or pathological changes in the heart (Tables II and III), or both; one case had a history of auricular

fibrillation up to the time of death but showed no demonstrable lesion. Thus, 47, or 84 per cent, may be regarded as having had impairment of cardiac function. Of this observation we shall have more to say later.

The main problem arising in connection with pulmonary embolism is the etiology of spontaneous venous thrombosis. Though our knowledge in this direction leaves much to be desired there are, without going into controversial points, several well established factors known to favor the process. Virchow¹⁵ emphasized the importance of slowing of the blood stream as a factor predisposing to *intra vitam* coagulation of blood. Cohnheim,¹⁶ and Ribbert^{17, 18} showed that injury to the lining of a blood vessel afforded a localizing influence favorable, if not essential, to the development of thrombus. Aschoff^{19, 20} determined a further condition, namely eddying of the blood stream, as propitious for the laying down of thrombus. Hueck,²¹ and Dawbarn, Earlam and Evans²² showed that an increase in the blood platelets synchronizes postoperatively with the period during which thrombosis is to be feared. There is now general agreement that two or more of these factors must operate in conjunction to be effectual; acting singly they are unproductive of thrombus. Besides these points it is common knowledge that thrombosis occurs with greater frequency beyond middle life than before, that it is prone to take place in the wake of operations, childbirth and acute infections.

From the present study it seems warrantable to emphasize another general principle concerning the occurrence of thrombosis, a principle that has received only casual attention in the literature, namely, that so-called spontaneous venous thrombosis has a high incidence in cases of actual or impending congestive heart failure. Virchow drew attention to a type of thrombus that developed in cachectic individuals, due, he thought, to a sluggish circulation, and he called it marantic thrombus. Welch²³ published a paper, "Venous Thrombosis as a Complication of Cardiac Disease." He reported 4 cases of his own and collected 23 more from the literature. Nearly all presented thrombophlebitis of the upper extremities with definite clinical manifestations. He was inclined to regard it as a relatively rare complication of heart disease, but it must be remembered he was dealing only with cases in which the thromboses had attracted attention during life, whereas in the majority of our own cases the thrombi

were incidental autopsy findings. Axhausen⁹ found 270 instances of venous thrombosis in 1472 autopsies on cases of heart disease. Rosenthal²⁴ reported 94 instances of thrombosis in 149 autopsy cases of "cardiac decompensation," but did not indicate the nature or location of the thromboses, whether in heart, arteries or veins. Oberndorfer²⁵ observed that out of 97 medical cases dying of pulmonary embolism 64 were ill with cardiovascular disease at the time of the fatal seizure. He also is indefinite as to the nature of the cardiovascular lesions and their relation to venous thrombosis. Bauer²⁶ says 95 out of 100 cases of postoperative thrombosis and embolism observed by him had hearts that were not normal. He believes an impairment of cardiac function to be an important factor in the etiology of venous thrombosis, but does not discuss the nature of the cardiac abnormalities or their relation to thrombosis. Ophüls and Dobson²⁷ report cardiovascular disease in 52 per cent of cases of thrombosis and embolism, but again details are lacking. Putnoky and Farkas¹⁰ found approximately 90 per cent of 91 cases of pulmonary embolism had cardiovascular disease and a large number showed fatty degeneration of the myocardium.

Our own figures show 36 instances of venous thrombosis in 83 autopsy cases of congestive heart failure and 49 instances of impaired cardiac function in 56 autopsy cases of pulmonary embolism. The dangerous type of venous thrombosis is that which develops without local signs and without demonstrable inflammation in the wall of the vein, and this type was observed 20 out of 83 times in association with chronic passive congestion of viscera, or anasarca, or both, due to heart failure. The same type of thrombosis was found 25 times in the other 484 autopsies of the series. Of these latter 25 cases, which constitute the bulk of Groups 2 and 3, 18 had evidence of cardiac inadequacy (see Tables II and III), though this was not the main cause of death. Thus in 46 cases of spontaneous venous thrombosis (with fatal embolus in 27) 20 were cardiac invalids and 18 more showed anatomical or functional changes in the heart.

What is the relation of cardiac incompetence to the development of venous thrombosis? To our knowledge this relation has never been defined beyond the general assumption that the circulation is slowed up. But what definite evidence is there that the velocity of flow in the veins is decreased when the function of the heart is impaired? To this question the work of Blumgart and Weiss²⁸ provides a definite

answer. These authors have shown that the circulation time is appreciably prolonged in cases of cardiac insufficiency, that the velocity of flow from peripheral veins to the right heart is definitely slower than in normal individuals, and that the venous pressure is increased. The observations of Blumgart and Weiss make it possible to postulate a slackened venous blood flow as a factor present in most of our cases of pulmonary embolism.

That the leg veins are the commonest site of thrombosis points still further to stasis as an important factor in the development of thrombosis. As Aschoff has pointed out, the veins of the lower extremities are the first to suffer a retardation of flow when the general circulation lags, partly because of the long column of blood in these vessels, partly because they may be compressed by Poupart's ligament, or in the case of the left common iliac vein, by the iliac artery. Other factors that tend to produce venous stasis in the lower extremities are increased intra-abdominal tension and immobilization of abdomen and legs. Of all operative procedures, incision through the anterior abdominal wall is the commonest to be complicated by pulmonary embolism. There were 6 instances of this complication in our series. Following laparotomy conditions are especially favorable for the development of thrombus in the leg or pelvic veins, probably because the venous return from the lower extremities is slowed up as the result of inertia of the abdominal musculature. The flow of blood up the inferior vena cava is retarded through diminution of the massaging effect of normal respiratory movements within the abdomen. If, in addition, the heart be embarrassed (as it was in 4 of our 6 cases), the venous return is still further retarded and stasis, then, unquestionably plays a major rôle. Fracture of the femur, which is not infrequently associated with pulmonary embolism (McCartney ²⁹), also has the effect of rendering leg and pelvis inert, thus favoring venous stasis. There were 3 cases of fractured femur complicated by fatal embolism in our series and in all 3 heart disease was present. There were also 2 instances of compression of the inferior cava by intra-abdominal tumors (Table II) and both suffered fatal embolism from thromboses developing below the point of compression.

Several clinical investigators (Walters,³⁰ Miller,³¹ Polak and Mazola,³² Boshamer,³³ Fonio,³⁴ and Wright³⁵), already convinced of the danger of venous stasis in postoperative cases, describe prophylactic measures aimed at maintaining adequate venous return from the

extremities. The administration of thyroxin to stimulate the circulation, elevation and massaging of the legs, application of heat and the encouragement of active movement are some of the measures employed in an attempt to prevent postoperative pulmonary embolism. Walters³⁰ reports encouraging results of such treatment at the Mayo Clinic.

Of the 56 cases that form the basis of this report only 11 had undergone operative treatment. Platelet counts were not made, but the factor of "tissue injury and the absorption of breakdown products" (cited by Dawbarn, Earlam and Evans²¹ as the probable excitant of the postoperative platelet rise) was not present in the majority of our cases.

The work of Hueck,²¹ and Dawbarn, Earlam and Evans,²² has served to focus much attention on a relation between increase of platelets and a tendency to thrombosis. It should be remembered, however, that age does not influence the postoperative platelet rise; it is just as marked in the child as in the adult, and yet the latter is much more prone to develop thrombosis. Dawbarn and his associates state: "If the two factors of stasis and a high platelet level be combined in a given patient it would seem that thrombosis may be expected. In elderly patients the factor of stasis is probably the more important." It should also be borne in mind that no one, with the exception of Brock,³⁶ seems to have made observations on the platelet reaction in actual cases of thrombosis. Brock states that he has seen thrombosis in cases where the platelet count showed only a moderate rise, whereas other patients with a much bigger rise showed no evidence of any thrombosis. Willinsky³⁷ studied the platelet reaction in a number of postoperative cases and in general confirmed the observations of Dawbarn, Earlam and Evans, but none of his cases had thrombosis.

A large number of workers have directed their attention to changes in the coagulability of the blood, believing an altered state of the blood to be the chief factor responsible for thrombosis. While not denying the possibility of this factor playing a rôle it seems to us the fact is too often overlooked that thrombosis is a process essentially different from *extra vitam* coagulation of blood. The two types of coagula are widely different in origin and structure, as has been shown by Zahn,³⁸ Eberth and Schimmelbusch,^{39, 40} Welch,⁴¹ Aschoff,^{19, 20} Apitz,⁴² and Dietrich.⁶ Dawbarn, Earlam and Evans,²²

while purporting to show a correlation between the platelet count and clotting time, observed that patients with very high or very low platelet counts often showed but slight variations from the normal clotting time. The parallel which they believe to have established is not a striking one and there remains room for doubt as to whether the rapidity of *extra vitam* clotting varies directly with the tendency to thrombosis, whatever be the influence of the platelets.

The observations of Lubarsch¹ and Aschoff^{19, 20} have done much to disprove the contentions of Welch,^{23, 41} Dietrich⁶ and others, that infection plays the most important rôle in the determination of thrombosis. In the majority of our cases the presence of infection could not be established as a definite factor. Furthermore, we sought to exclude from the present study those cases in which thrombosis arose as the result of suppurative lesions in relation to veins. Microscopic examination of the thrombosed veins failed to show signs of an active inflammatory reaction, thus affording further evidence of the absence of infection.

SUMMARY AND CONCLUSIONS

Pulmonary embolism is to be observed in approximately 10 per cent of autopsies upon adult individuals and is much commoner in medical than surgical cases. More than half of these cases show infarcts of the lungs and in nearly all there are repeated embolisms occurring over a period of hours, days or weeks. Thromboses of leg and pelvic veins are the chief source of dangerous pulmonary emboli. Such thromboses commonly develop without clinical manifestations and show no evidence of active inflammation in the adjacent vein wall. The dangerous type of thrombosis has a high incidence in cases of cardiac insufficiency. From the work of Blumgart and Weiss it is evident that the flow of blood in the veins is slowed when the heart's function is impaired, and slowing of the blood stream is known to favor thrombosis. Other factors seeming of lesser significance in our series of cases, we regard circulatory embarrassment as of prime importance in the etiology of venous thrombosis and pulmonary embolism. A high percentage of postoperative fatalities from pulmonary embolism show evidence of minor degrees of cardiac incompetence.

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THE AMERICAN JOURNAL OF PATHOLOGY

VOLUME X

MARCH, 1934

NUMBER 2

HYPERACTIVATION OF THE NEUROHYPOPHYSIS AS THE PATHOLOGICAL BASIS OF ECLAMPSIA AND OTHER HYPERTENSIVE STATES *

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An excessive infiltration of the neurohypophysis by epithelial elements, bearing a certain resemblance to the cellular invasion of a malignant tumor, appears to have been first mentioned by Thom in 1901,⁴⁵ since when the condition has been observed and commented on from one aspect or another by many others. There has, however, been a difference of opinion regarding the precise nature as well as source of origin of the inwandering cells, and still less agreement as to the meaning of the process. Indeed, it has not been generally assumed to have any physiological or pathological significance.

In the lower animals a patent cleft, the relic of Rathke's pouch, divides the epithelial portion of the pituitary body into a bulky pars distalis and a thin pars intermedia, which serves closely to envelop the pars nervosa proper. The posterior lobe or neurohypophysis is thus composed of two easily recognizable but mechanically inseparable portions.†

* These studies, made in the Surgical Laboratory of the Peter Bent Brigham Hospital with the assistance of Dr. Louise Eisenhardt, were the basis of the first lecture before the Medical Research Society delivered at University College, London, November 2, 1933.

† Closely embracing the pituitary stalk and lower tuber, both in animals and in man, is a tongue-like prolongation of the epithelial lobe known as the pars tuberalis, of whose independent secretory function even less is known than of the pars intermedia. The chief difficulty encountered by those who have attempted, by study of its extracts, to determine the separate function of the pars tuberalis has possibly lain in the fact that the large venous trunks which pass through it contain variable amounts of the secretory product both of pars distalis and of pars intermedia in the process of transport to the tuberal nuclei.

Received for publication December 12, 1933.

In the higher anthropoids and in man, on the other hand, because of the practical disappearance of the cleft there exists no such clear anatomical distinction between pars distalis and pars intermedia. Consequently, many writers (*e. g.*, Plaut³⁶ in 1922, Erdheim¹⁷ in 1925, Dayton¹⁴ in 1926, Benda⁵ in 1927, and Kraus²⁷ in 1928) have expressed the belief that the latter has become so rudimentary or vestigial it is futile to consider the two epithelial parts of the human gland other than as a whole. This has been particularly emphasized during the past several years by Berblinger,^{6,7} who distinguishes glandular hypophysis from neural hypophysis but disclaims any recognizable subdivisions of the former. Hence, in accordance with this view, any cells that wander into the pars nervosa must come from the pars distalis or anterior lobe proper.

Several of the authors who adhere to this opinion, more especially Kraus and Berblinger, have examined large numbers of human glands removed at autopsy and have made detailed estimates of the relative percentage of basophilic elements present in the pituitary body as a whole under various conditions of disease. Berblinger's computations of an increase or diminution of these elements are based on their relative number, irrespective of the lobe in which they occur. Kraus,²⁷ on the other hand, lists those of anterior and posterior lobe separately and it would appear from his tables that the pars nervosa often harbors basophilic elements, particularly in association with what he calls the hypersthenic constitution.²⁸

However, from my reading of their papers it does not appear that either of these distinguished writers on the subject looks upon the basophilic infiltration of the pars nervosa as anything more than a fortuitous overflow of these elements from the pars anterior. Berblinger, indeed, emphatically insists that pituitary disease is entirely an anterior lobe problem. Just why the acidophilic elements of this lobe, which in man are heavily massed in the region adjacent to the site of the original cleft, fail similarly to infiltrate the nervous tissue is not explained.

A contrary view, with which the writer sides, is held by another group (*e. g.*, Tölken⁴⁶ in 1912, Schönig⁴⁰ in 1926, Lewis and Lee³⁰ in 1927, Marburg³¹ in 1929, Biedl⁸ in 1929, Aschoff² in 1930, Rasmussen³⁸ in 1930, Orlandi³⁴ in 1930, Guizzetti²⁹ in 1933). While granting its relatively inconspicuous nature in man, most of these authors nevertheless maintain that the pars intermedia is anatomi-

cally recognizable by the distinctive character of its cells, which acquire basophilic granules in the process of maturation and migrate into the pars nervosa. Though the basophils arising from this source possibly tend to be somewhat smaller, to have a more pyknotic type of nucleus and a less heavily granular and less abundantly vacuolated cytoplasm than the basophilic elements of the pars distalis, in the terminal stages of ripening the basophils from either source are morphologically indistinguishable.

These differing points of view regarding the source of origin of the basophilic elements occasionally observed in the pars nervosa would seem to be of less importance than the physiological significance of the process; and it does not appear to have been suggested, or at least not to have been emphasized, by either party in this contention that *the degree of basophilic infiltration may represent a measure of neurohypophysial activation*. It is proposed herein to lay stress on this fundamentally important point.

That the posterior lobe contains an active principle, not found in the anterior lobe, has been known since Howell's discovery in 1898 of a pressor substance in its extracts. However, all posterior lobe extracts obtained from the lower animals are necessarily products of both pars intermedia and pars nervosa. And since it is inconceivable that the neural tissue composing the tubero-infundibular apparatus is capable independently of elaborating a hormone, the active principle in extracts of the lobe must obviously be derived from its cellular investment. It certainly could not come from the pars distalis, for in the lower animals from which these extracts are customarily made the two lobes of the gland are readily separated.

Herring²¹ was the first to study and describe the peculiar manner of posterior lobe secretion. In the gland of the cat he observed the casting off from the pars intermedia of cells which in their passage through the pars nervosa become transformed into hyaline-like masses. These masses of secretory product, in favourable preparations, not only are discernible in the posterior lobe of normal glands but may be increased visibly under certain experimental conditions such, for example, as after a preceding thyroidectomy.²² While Herring's conception of the process has been looked upon with skepticism in many quarters and may need some slight reinterpretation, a number of authors (*e. g.*, Sharpey-Schafer,⁴¹ Cushing and Goetsch,¹³ da Costa,¹⁰ and Remy Collin⁹) have agreed with him in

all essential points. His views unquestionably furnish the only satisfactory explanation of the normal manner of neurohypophysial activity.

As mentioned in the introductory paragraph, a massive infiltration by basophilic elements is not infrequently seen in the posterior lobe of man. However, not even by those who believe in a persistent *pars intermedia* and look upon the invading elements as derived from it does the suggestion appear to have been made that the degree of infiltration is a measure of functional activity and that an excess of posterior lobe (*i. e.* *pars intermedia*) secretion should be recoverable from glands in which the process is marked.

It has been pointed out separately by Erdheim, Tölken, Kraus and Berblinger that an increase of basophilic elements in the gland as a whole is an accompaniment of advancing years of life when naturally enough it is often associated with atherosclerosis and renal disease. They appear, however, to regard the process as merely coincidental with these disorders and do not look upon it as in any sense an aetiological factor in their production, unless such a statement has been overlooked. In his recent monograph Berblinger⁷ emphatically states (page 936): "Neither Hoeppli nor I ever claimed that the increase in the basophilic cells represents a pathological finding in the hypophysis but on the contrary, we regarded the cellular variation as a reaction that supposedly bears some sort of relationship to the altered renal activity."

Of the several pathologists who have dealt with the subject Skubiszewski⁴² (1925) appears to have been the only one to have grasped the idea that posterior lobe basophilia might be an indication of hyperfunction. He expressed the belief that the cardiac hypertrophy and diuresis accompanying chronic interstitial nephritis might thus be accounted for. This view appears to have been based on the assumption that the posterior lobe principle had a diuretic rather than an antidiuretic action. But however this may be, the time was not ripe for such an interpretation and Berblinger promptly opposed it on the ground that in uraemia with lowered diuresis the same picture is particularly frequent. As a matter of fact, only in the past few years through the discovery of a posterior lobe-like substance in the blood in certain hypertensive states could the full significance of hyperactivation of the posterior lobe by the invading elements have been thoroughly grasped.

1. NEUROHYPOPHYSIAL ACTIVATION IN PITUITARY BASOPHILISM

What has led to a revival of interest in this particular matter has been the postmortem disclosure, in a case of what has been termed pituitary basophilism,¹¹ not only of a definite basophilic adenoma in the pars distalis, but of an excessive invasion of the pars nervosa by elements of the same type (Fig. 1). This strongly suggested a dual source of the symptom-complex as partly anterior hypophysial and partly posterior hypophysial. For in these clinical states not only is there evidence of gonadal dysfunction but the adiposity, glycosuria, pigmentation of the skin, hypertension and ultimate atherosclerosis might well be ascribed to hyperfunction of the posterior lobe.

This disclosure naturally led to the re-examination of sections of the pituitary glands from the known victims of the disorder obtained from various sources; and though in the single sections from the Anderson case, the Parkes Weber case, and one or two others, no notable basophilic invasion of the posterior lobe is seen, it is very marked in the gland from the Raab-Kraus case, of which Professor Kraus has kindly submitted four sections. Indeed, on further study of this case, the interpretation of which has been thoroughly discussed in the literature both by Kraus²⁶ and by Raab,³⁷ it is my impression that the tumor is an actual adenoma of the pars intermedia (Fig. 2).

While this interpretation of the lesion is not in accord with that held by Dr. Kraus, to whose opinion and wide experience I should naturally wish to defer, certain reasons in its favour may be given. Not only is a rich basophilic invasion of the pars nervosa taking place from the periphery of the adenoma, as he has clearly pointed out, but there is also a tendency in this direction in areas remote from the tumor where the investing pars intermedia is separated from the pars anterior by a large Rathke's cyst (Fig. 3). It would seem, therefore, that the cells must come from the pars intermedia rather than the pars distalis from which, indeed, the adenoma itself is sharply delimited. A further reason lies in the fact that an adenoma of similar sort, unmistakably from pars intermedia, has been observed in a fatal case of eclampsia to be described (p. 156).

The vasculo-renal changes incidental to old age have, as already stated, been shown to be accompanied by an increase of basophilic elements of the pituitary body as a whole. But since hypertension

and atherosclerosis of pituitary basophilism occur in young persons, it was natural to assume that the basophilic adenoma was the causal agent rather than a resultant effect. What is more, since extracts of the posterior lobe alone contain a demonstrable pressor substance, the conclusion was inescapable that the posterior lobe basophilia was the important factor in the hypertension, rather than the numerical increase of these cells in the pars distalis.

2. NEUROHYPOPHYSIAL ACTIVATION IN ECLAMPSIA

We may now turn to evidence from another source. Several years ago (1918) it was pointed out by Hofbauer²³ that the diminished output of urine, the oedemas, convulsions and vascular hypertension characterizing eclampsia strongly suggested an intoxication by excess of posterior lobe secretion. In pursuit of this hypothesis Anselmino and his collaborators^{1, 2} have found an antidiuretic substance in the blood of patients with eclampsia, and also a pressor substance in all instances when systolic blood pressures of 180 or over were a feature of the syndrome.

In view of these interesting observations it was anticipated that the same excessive infiltration of the pars nervosa, which was so striking in the case of pituitary basophilism with hypertension and nephrosclerosis, might also be present in the pituitary glands of patients with eclampsia.

A hint that this idea might be worth pursuing was afforded by the sections of a gland in which such an infiltration had been observed (Fig. 4). The specimen came from the Boston Lying-In Hospital and the sections have been filed in our laboratory since 1921, when Percival Bailey⁴ was in search of a tinctorial method of distinguishing the granules in basophilic and acidophilic cells. There unfortunately is no history of the specimen and it is not definitely known to have come from an eclamptic patient, but considering its source the probabilities are that it did.

Since Erdheim and Stumme's classical paper¹⁸ (1909), in which the pregnancy cells were first described, most pathologists who have studied the pituitary body in pregnancy have been more interested in the condition of the glandular than of the neural hypophysis. Though Erdheim had been among the first to describe the basophilic invasion of the posterior lobe no reference is made to its occurrence in any of the eighteen cases of eclampsia that he and Stumme in-

cluded in their important monograph. On the other hand, in three of the twenty-five glands in their control series (two cases of nephritis and one of biliary carcinoma) such an invasion is specifically mentioned. The professor of pathology in another university has kindly forwarded for study single sections of the pituitary bodies from eleven cases of eclampsia in his collection. Five of them fail to show the posterior lobe at all, and in none of those that happen to retain portions of it does any active cellular invasion appear. All this would seem to constitute overwhelming evidence against the view that posterior lobe activation by basophils might be a factor in the toxæmias of pregnancy.

Not only are pituitary glands of eclampsia difficult to come by, but it would appear that the "toxicozes of pregnancy" and "eclampsia" are exceedingly vague terms in obstetrical parlance. During the past few months we have succeeded in securing nine uncut glands supposedly from fatal cases of the disorder. While not all of them show basophilia of the posterior lobe, some of them do, and in a few instances it is excessive. To a consideration of these cases we may now turn.

For the first two specimens which came from the pathological department of the Boston Lying-In Hospital I am indebted to my colleague, Professor S. B. Wolbach.

CASE 1. The patient, 28 years of age, had been under treatment for advanced diabetes mellitus for a period of 6 years. She was sent to the hospital a few hours after a normal parturition because of a sudden convulsion with subsequent coma. The systolic blood pressure was 170; the urine showed large traces of albumin and the blood a marked hyperglycaemia. She was twice subjected to plasmapheresis with fatal issue. While the diagnosis was "puerperal toxæmia with convulsions," there was some question as to whether the death might not have been due to diabetic coma.

The serial sections of the *pituitary body* show no posterior lobe invasion whatsoever.

The history of the second case from the same source is as follows.

CASE 2. On March 29, 1931, an 8 months pregnant primipara, aged 34 years, was brought to the hospital in a comatose condition after having had three consecutive convulsions. The urine drawn by catheter was small in amount and showed a large trace of albumin. Plasmapheresis was performed and she was given hypertonic glucose and saline solution. The blood pressure was variable, the highest reading having been 190/100. The lowest reading of 50/38 was taken shortly before death, which occurred on the day after admission, the patient never having regained consciousness.

The postmortem examination showed: an 8 months undelivered fetus, acute tubular nephritis, slight fatty infiltration of the liver with central congestion and necrosis, acute toxic splenitis, pulmonary congestion and oedema with (?) terminal bronchopneumonia, generalized slight arteriosclerosis, and follicular desquamation of the thyroid.

On its removal in 1931 the *pituitary body* had been cut sagittally in halves, one of which was preserved in alcohol, the other in Zenker's fluid. On being sectioned serially the *anterior lobe* shows a great abundance of basophils, often in large clusters, some of almost adenomatous-like character. There is no necrosis, scarring or round-cell infiltration.

In the *posterior lobe* (evidently cut into and partly lost in process of removal) the distinction between pars distalis and pars intermedia is easily drawn by long, narrow, colloid-containing cavities representing the original cleft. In the more lateral regions the cleft may be followed all the way through to its open mouth in the dura. Because of this separation it is difficult to imagine that the fairly abundant basophilic infiltration of the posterior lobe (Fig. 5) represents an overflow from the pars distalis.

The colloid-filled cleft extends from the base of the gland four-fifths of the way up toward the stalk. Above this level the distinction between pars distalis and pars intermedia is less clear. Below this level signs of reactive hyperplasia of the pars intermedia are everywhere evident; the acini have increased in number and ripened basophilic elements are being cast off to invade the adjacent nervous tissue. Fortunately the hyaline masses have not been wholly dissolved out of the tissue and the pars nervosa is everywhere heavily charged with them, large accumulations being present in certain regions (Fig. 6).

For the next three specimens to be described thanks are due to Dr. G. Elliott May of the Boston City Hospital.

CASE 3. On Jan. 30, 1933, Mrs. A. F., a primipara 42 years of age, first consulted Dr. May when about 7 months pregnant. For 2 months she had been having frequent vomiting attacks, which were ascribed to indigestion from which she had suffered for years. Latterly she had voided infrequently and for the past week only very small amounts.

She was emaciated and dehydrated, having a dry, coated tongue, slight oedema of the ankles and a blood pressure of 160/100. The urine showed a trace

of albumin, a few red cells and occasional granular casts. Test of renal function showed it to be low; the non-protein nitrogen was 36 mg. per cent. She was immediately sent to the hospital with the diagnosis of toxæmia of pregnancy and hyperemesis gravidarum.

During the next week under forced fluids she improved greatly. The urine increased in amount, the oedema disappeared and the vomiting ceased. Her blood pressure, however, progressively rose to 184/128 by February 7th, on which day she began having labour pains. Of these she complained so bitterly she was given phenobarbital and scopolamine in small doses. Later in the day she passed into a coma from which she never aroused. On the afternoon of February 8th she was delivered normally, with the aid of an injection of pituitrin, of a still-born foetus. The systolic blood pressure dropped from 182 to 138 and 2 hours later to 90/70. She remained comatose, in spite of all efforts to relieve the condition, until her death on the afternoon of February 10th.

The postmortem examination disclosed an apopleptic clot in the right frontal lobe of the cerebrum, a duodenal ulcer, and multiple small abscesses of liver and kidneys. There was no atherosclerosis. The diagnosis was "non-convulsive eclampsia without typical autopsy findings."

The *pituitary body* had been cut in two in a sagittal plane, the stalk having been destroyed in the process; one-half had been fixed in Zenker's fluid, the other in formalin. Serial sections of each block were made in the vertical plane of the original cut.

The *pars distalis* shows near its anterior edge an irregularly marginal area of necrosis about 3 mm. in diameter. Infiltration with polymorphonuclear leukocytes has begun to take place in the necrotic area, which is encircled by alveolated clusters of basophilic cells more sharply outlined than usual because of their separation by oedematous strands of interstitial tissue.

Throughout the *pars distalis* basophils far outnumber the acidophilic elements, the latter being largely confined to a broad juxtanuclear crescentic strip. Many large chromophobe elements (gestation cells?) are scattered through the lobe and one gains the impression that they are ripening into pale staining basophils.

The *pars intermedia* is clearly separated from the *pars distalis*, throughout most of the sections, by the overabundance of colloid in the cleft. There is an extensive posterior lobe invasion (Fig. 7) by basophilic elements from the *pars intermedia*, more particularly from the lower third of the cleft. The column of cells extends in the usual conical fashion halfway through the lobe.

Throughout the *pars nervosa* the hyaline masses happen to have been unusually well retained in the interstices of the neural tissue

(Fig. 8), many of them adjacent to the tongues of the still living cells showing ghosts of nuclei. The hyaline masses can be followed easily as they stream toward the direction of the stalk. The abundant hyalin (colloid) in the cleft appears to come from the same cellular elements. It can be seen emerging from the mouth of the cleft into the subarachnoid spaces.

In all three of the foregoing cases the gland, before it was received, had been divided on a sagittal plane, the two halves having been placed in different fixatives. This procedure, for reasons given elsewhere,² adds difficulties of interpretation to the study of the serial sections from the loss of topographical relations. Wishing to obtain an entire gland with its hypothalamic attachment intact, Dr. May kindly notified me of the autopsy on the following case and I was permitted to remove and preserve the block of tissue in the desired way.

CASE 4. The patient, an exceedingly adipose multipara 38 years of age, was admitted to the Boston City Hospital June 6, 1933. Three years before she had been attended by her local physician in her ninth pregnancy. At that time she had a normal parturition, though she was found to have a blood pressure of 180/100. As this condition subsequently persisted, it was looked upon as an essential hypertension.

During this, her tenth pregnancy, the systolic pressures had varied from 200 to 220. She latterly had been having much nausea and vomiting with swelling of the hands and feet.

Before her admission she had been in labour for several hours with a breech presentation and, becoming hysterical, she was finally taken to the hospital. There she was found to have a blood pressure of 210/120, going up to 260/120 during her pains. The delivery of the child was tardy and subsequently the mother passed into a comatose state without convulsions and died in a few hours. The clinical diagnosis was "toxaemia of pregnancy."

The autopsy showed very little apart from a moderate cardiac hypertrophy and dilatation, slight atherosclerosis, fatty infiltration of the liver and acute pulmonary congestion.

The *pituitary body*, a large, succulent gland (not separately weighed) with its stalk, tuber and block of the hypothalamic region, was removed in one piece (Fig. 9), fixed in formalin, serially sectioned in the horizontal plane and stained with haematoxylin and eosin.

The posterior lobe in its lower portion shows a cellular invasion by basophilic elements that almost surround its circumference (Fig. 10). A large excess of colloid in certain parts of the cleft has broken widely

into the pars distalis. In many areas the infiltration is massive (Fig. 11) and strands of normally staining basophils can be followed well up into the stalk (Fig. 12). The holocrine secretion has been well preserved in between the infiltrating tongues of viable cells (Fig. 13). The tuber is broken up into widely opened spaces as the tip of the infundibular cavity is approached. This, as usual, shows a highly defective endymal cuticle.

The story of Dr. May's third case is briefly as follows.

CASE 5. On Aug. 12, 1933, a 23 year old Polish woman, about 7 months pregnant, who had had no prenatal care, was admitted to the Boston City Hospital with the story that she had recently shown some swelling of the face and ankles, and for 24 hours had been having a series of convulsive seizures. She was unconscious on admission and about 3 ounces of urine were obtained by catheter, showing a heavy trace of albumin, hyaline and granular casts and red cells. Her blood pressure was 170/110. She remained in deep coma in spite of treatment and died 36 hours later.

The postmortem examination showed lesions in the liver and kidneys typical of eclampsia. There was also an intense venous congestion of the cortical vessels of the brain with a small subarachnoid hemorrhage over the right occipito-parietal region.

The *pituitary body* with the hypothalamus had been removed in a single block, fixed in formalin and forwarded for study. The gland was large; both stalk and tuber were swollen and succulent. After serial sectioning in the horizontal plane not only are basophiles found to be abundant in the pars distalis, but from the pars intermedia two cones of these same elements project into the pars nervosa (Fig. 14), from one of which ripened cells can be traced well into the center of the lobe. The pars intermedia in other regions shows an abundance of Rathke's cysts lined by ripened basophiles (Fig. 15) which have broken into the cysts as well as the cleft and are scantily invading the pars nervosa.

In the Kraus-Raab case of pituitary basophilism, as previously stated, there was found what I have ventured to interpret as a basophilic adenoma of the pars intermedia (*cf.* Fig. 2). Some hesitation was felt in regard to this for the reason that no such adenoma of this epithelial zone had been definitely described. However, the disclosure of a similar lesion in the gland of a patient with eclampsia makes the given interpretation of the case seem the more probable.

Through my one-time pupil, Dr. Benno Schlesinger, some inquiries were made regarding the prevalence of eclampsia in Vienna. Desiring to interest Professor Erdheim in the subject at hand and with the hope that his old eclampsia sections might be gone over to see what proportion of them showed invasion of the type in question, a photomicrograph of one of our sections was sent in illustration of what was to be looked for. He replied that he had never seen any corresponding degree of "spreading out" of basophilic cells in the posterior lobe, except in the glands of old persons. Unfortunately his old slides had been thrown away and for years he had had no opportunity further to pursue his studies of pregnancy.

Dr. Schlesinger made further inquiries and learned at the Allgemeines Krankenhaus that they do not have more than one or two fatal cases of eclampsia each year. He subsequently, from another source, had the good fortune to secure and forward to me the gland to be described.

CASE 6. The patient, a primipara aged 37 years, was admitted to the Brigittaspital of Vienna May 2, 1933. She had marked hypertension, the systolic registrations ranging between 210 to 180 during the next 10 days. On May 13th she had six convulsive seizures and at 6 P.M. the child was delivered by forceps extraction. In spite of stimulants she failed to rally and died 8 hours later.

The postmortem examination disclosed a "gray and fragile" liver, a parenchymatous and fatty degeneration of the kidneys, excentric hypertrophy of the left ventricle, acute oedema of the lungs, and oedema of the leptomeninges.

The *pituitary body* was large, ovoid, and weighed *circa* 960 mg. The prominent posterior lobe had been slightly damaged in removal; there was an obvious extrusion of a large hyaline globule in the cleft between the posterior and anterior lobes.

Serial sections on a horizontal plane were cut at 8 microns, every tenth section being mounted and stained with haematoxylin and eosin. The first thing noticeable is the large, full *pars distalis* which shows no areas of necrosis or round-cell infiltration. Basophilic elements abound, many of them in large clusters. The transverse cleft is distended with colloid which has burst through into the meninges. It cleanly separates *pars distalis* from *pars intermedia* (Fig. 16). The posterior lobe at this level is defective but an extensive invasion from *pars intermedia* is clearly apparent (Fig. 17).

The cells from this low-level invasion pass upward and backward toward the posterior portion of the *pars nervosa* where they become

merged with a large, sharply defined cellular mass (Fig. 18). This globular lesion is readily visible to the naked eye from the 8 micron sections No. 720 to 2250 (Fig. 19), its maximal diameters being about 3 by 4 mm. It proves on higher magnification to have the architectural features of an adenoma (Fig. 20) and its component elements are unmistakably fully ripened basophilic cells (Fig. 21).

The glands of the two following cases were received through the courtesy of Dr. C. B. Courville of Los Angeles.

CASE 7. The patient, an obese woman aged 35 years, and 7 months pregnant, was admitted to the Los Angeles General Hospital May 13, 1933, having had three convulsions the previous day. She had had no prenatal care. There was some oedema of the ankles, feet and face. The urine showed a large trace of albumin and finely granular casts. The blood pressure was only 130/90. On May 18th she was delivered of a premature child. On May 19th she became comatose with Cheyne-Stokes respiration and was found to have a bilateral papilloedema. On May 22nd she died. The case was looked upon as one of typical postpartum eclampsia.

At autopsy changes in the liver and kidneys were found consistent with eclampsia and there were in addition multiple focal haemorrhages in the brain.

The *pituitary body* shows very little change. Posterior lobe invasion is slight, occurs in one small area only (Fig. 22) and there is no colloid in the cleft.

In the following, the second of Dr. Courville's cases, there was doubt of the diagnosis.

CASE 8. The patient, a multipara 7 months pregnant, was admitted to the Los Angeles General Hospital on June 15, 1933, in status epilepticus from rapidly recurring right-sided fits. She had been known to have convulsions previously of Jacksonian type beginning on the right side. The blood pressure was 165/100. The urine showed a trace of albumin and a few casts. The cerebrospinal fluid was blood-tinged and under tension. A diagnosis of subdural haemorrhage was made and an operation performed without disclosing a clot.

The autopsy showed cerebral oedema with petechial haemorrhages, a thickened arachnoid and an apparent thrombosis of the left middle cerebral artery. The case was looked upon as "atypical eclampsia."

The *pituitary body* on section shows (Fig. 23) only a very slight degree of posterior lobe invasion in one place. There is certainly no excessive basophilia in either anterior or posterior lobe.

The records and specimen from the last of the cases have been kindly forwarded by Dr. Frank Forry of the Indiana University Medical School.

CASE 9. The patient, 44 years of age, was an obese multipara in the 8th month of her ninth pregnancy. She had been known to have hypertension for several years. She was admitted to the hospital Aug. 16, 1931, in deep coma with cyanosis. There was oedema of the extremities. The urine showed a large trace of sugar, albumin, red cells and granular casts. The blood pressure, taken frequently, ranged from 238/130 to 190/110. She was spontaneously delivered of a still-born child and died 3 days after admission in a state of hyperthermia (107° F).

At autopsy focal necroses of the liver, chronic nephritis and bronchopneumonia were found. There was no question of the diagnosis of eclampsia gravidarum.

The *pituitary body* shows a massive posterior lobe invasion, as heavy as that shown in Figure 5. The gland unfortunately was fragmented in removal and the sections stain so feebly the photomicrograph is not worth reproducing.

Summary

Briefly summarized, six of these nine cases (Nos. 2, 4, 5, 6, 7 and 9) were typical of eclampsia and in the four (Nos. 2, 4, 6 and 9) that had shown marked hypertension an excessive basophilic invasion was present; Case 5 showed only a moderate invasion with the systolic pressure not above 170, and in Case 7 there was no hypertension and very slight invasion.

In the other three cases (Nos. 1, 3 and 8) the diagnosis of eclampsia was questionable or the condition atypical. There was no invasion in Case 1 with a systolic pressure of 170, and in Case 8 with a pressure of 165 it was slight. In Case 3, on the other hand, with a pressure of 184, the posterior lobe basophilia was marked. In all the cases, therefore, in which blood pressure registrations were 180 or over, there was marked basophilic infiltration of the posterior lobe. It will be recalled that Anselmino and Hoffmann found a pressor substance in the blood of eclamptics only when systolic pressures exceeding 180 were recorded.

3. ESSENTIAL HYPERTENSION IN THE PRIME OF LIFE

If I am correctly informed, it is generally recognized by obstetricians that when the toxæmias of pregnancy are accompanied by hypertension their victims are apt to retain an abnormally high blood pressure which is likely to be increased in each subsequent period of child-bearing. Alongside of this goes a tendency toward adiposity, examples of such a sequence being given by Cases 4 and 9 in the preceding series. So-called essential hypertension, however, is a common disorder by no means limited to such a small group, for it may victimize women who have never borne children and no less frequently men in the prime of life.

The postmortem examination on such cases often fails to show any satisfactory explanation for the patient's death. The usual finding on which the pathological diagnosis is based is a chronic progressive renal lesion characterized by hyalinoid thickening of the terminal arterioles. While these vascular changes may be more pronounced in the kidney than elsewhere and may possibly first be detected there, the process nevertheless is universal and similar arteriolar changes are found in all other organs. This was clearly pointed out sixty years ago by Gull and Sutton, whose important studies were the starting point of the vast amount of work that has since been done on arteriosclerosis and hypertension. Nevertheless, many clinicians are still inclined to regard essential hypertension (the "hyperpiesia" of Clifford Allbutt) as primarily a nephrovascular disorder, in view of the presence of albumin and casts in the urine.

While Gull and Sutton admitted complete ignorance as to the cause of their "arterio-capillary fibrosis" other than that it was common in old age and premature senility, it would have interested them to know that posterior lobe extract exerts its constricting effects on the peripheral arterioles and capillaries where the pathological changes they described primarily appear. What is more, as pointed out by Professor Harold E. MacMahon, precisely the same renal lesion, variously called progressive vascular nephritis and malignant nephrosclerosis, may be seen to follow both hyperpiesia and pituitary basophilism, and it is quite possible that the more acute renal lesions of eclampsia are of the same order.

In view of what has gone before, it was natural enough to suspect that posterior lobe basophilia might also accompany these conditions

of so-called essential hypertension. The first opportunity to examine the pituitary body from such a case in the desired way was afforded by Dr. George Hass, the resident pathologist of the Peter Bent Brigham Hospital, who removed the gland and anterior hypothalamus in one piece from the body of the man whose story follows.

CASE 10. Edward M., aged 45 years, a negro chef of good family history and exemplary habits, entered the medical wards of the hospital Feb. 8, 1933, complaining of precordial pain, shortness of breath, inappetence, and recent loss of weight. For 2 years he had been having morning headaches regressing during the day; for 3 months dyspnoea on exertion, often associated with substernal pain radiating to the left shoulder and ceasing abruptly; also attacks of nocturnal dyspnoea with productive cough; for 2 months transient attacks of blindness in the right eye, lasting a few hours; for 2 weeks occasional slight epistaxis.

The physical examination revealed a cardiac enlargement and an expanded aorta (shown by the X-ray), with soft systolic murmur and accentuated second sound. The blood pressure was high, varying around 230/160. The urine showed the slightest possible trace of albumin, an occasional red blood corpuscle, rarely a hyaline cast. The Wassermann reaction was positive for the blood (repeated), negative for the spinal fluid. There was no history of a syphilitic infection.

He was abundantly studied by many observers during the following 6 weeks with the diagnosis of syphilitic aortitis chiefly favoured, though some thought it was coronary disease, others a nephrovascular disorder. He had occasional attacks of severe pain, substernal or epigastric, during which his blood pressure would usually fall, on one occasion to 135/80. For these attacks he was given nitroglycerine and often required morphia.

In the early morning of March 25th he was taken with a typical attack of agonizing epigastric pain, which sedatives failed to relieve. This continued during the day, with periodic vomiting and frequent watery bowel movements containing blood. His blood pressure gradually fell to low levels, he became dyspnoeic, and died 24 hours later.

The postmortem examination showed a moderate cardiac hypertrophy, a slight degree of atherosclerosis, a progressive vascular nephritis and acute haemorrhagic colitis. It otherwise was essentially negative. There was nothing to support the clinical diagnosis of luetic aortitis, coronary thrombosis or myocarditis, and no cause for the "anginal" attacks was apparent.

Grossly the *pituitary body* was small, concave above, and its two lobes easily distinguishable, the posterior lobe being unusually prominent. Serial sections were taken through the entire block, including the hypothalamus, from below upward.*

The *pars distalis* shows no discernible abnormalities. The clusters of basophils are as usual principally disposed toward the anterior

* The sections from this case were the basis of a recent paper on the secretory activity of the two lobes of the gland and manner of their discharge.¹²

surface of the lobe and are not pathologically numerous. The acidophils are chiefly massed in the deeper portions of the lobe.

The *pars intermedia*, despite the almost total absence of a cleft, is clearly distinguishable from the *pars distalis* by the well marked limiting zone of basophilic elements which almost everywhere, even up to the root of the stalk, are actively invading the *pars nervosa*, here and there sending heavy wedge-shaped columns of cells deeply into the lobe (Fig. 24).

The *arterioles* encountered in the sections of the hypothalamus show precisely the same changes as those affecting the vessels of the kidneys, so that the process is a general rather than a local one. Numerous minute capillary haemorrhages have occurred in the *pars nervosa*, stalk and tuber.

This case is one of several in which very similar conditions have been found. The patients have usually been of middle age, often obese, have shown marked vascular hypertension, enlargement of the heart, traces of albumin with a few casts, and rare renal elements in the urine. They have usually succumbed with symptoms of acute pulmonary oedema. The postmortem examination has shown malignant nephrosclerosis with cardiac hypertrophy and a more or less marked atherosclerosis. Fatty infiltration of the liver has been common, also macular or ulcerative lesions of the gastro-duodenal mucosa.

4. HYPERTENSION WITH ATHEROSCLEROSIS IN THE AGED

These are the conditions in which an increase of basophilic elements sometimes invading the posterior lobe have already been described by Kraus, Berblinger, Erdheim and others. Nowhere, however, does it appear to have been suggested that the cellular invasion of the neurohypophysis was an indication of posterior lobe activation that might be the primary factor in the hypertension, causing in its turn the progressive vascular and renal changes so frequent in aged persons.

The cases are so common specific examples need scarcely be given. Not only have several instances been met with in our own series but during the past few months, since local attention has been drawn to the matter, some of the younger pathologists in the several hospitals associated with the Harvard Medical School have begun routinely to study the *pituitary body* in all autopsies. Some of them have brought

specimens showing marked basophilia of the posterior lobe. Good examples of massive invasion occur in the following two cases submitted by Dr. John I. Bradley of the Massachusetts General Hospital.

CASE 11. The patient, a 60 year old labourer, had been known to have a high blood pressure for some time before his admission to the hospital on Sept. 21, 1932, following a cerebral accident. Though conscious and alert, his speech was thick and unintelligible. The blood pressure was 180/110 and the eyegrounds showed moderate tortuosity and sclerosis of the arteries. On the morning following his admission he suddenly became unconscious, the blood pressure fell to 60/50, and the body temperature rose to 108.2° F just before death.

The autopsy showed hypertrophy and dilatation of the left ventricle with marked generalized atherosclerosis. A thrombus was found occluding the basilar and right vertebral arteries, causing an infarct of the pons and multiple organized infarcts of the basal ganglia. There was also a pulmonary infarct with secondary oedema and congestion. The posterior hypophysis shows a marked basophilic invasion (Fig. 25) with distention by colloid of the adjacent Rathke's cysts.

CASE 12. A 67 year old multiparous Irish housewife was admitted to the hospital on April 1, 1933, because of intermittent vaginal bleeding for the preceding few weeks.

Examination showed an obese, arteriosclerotic woman with a blood pressure of 190/100. There was some swelling of the ankles and a slight trace of albumin in the urine without casts, and the phthalein excretion was 50 per cent in an hour. The cause of her complaint was found to be a large cervical polyp, and her hypertensive disorder was thought to be sufficiently well compensated to justify the risk of surgical intervention.

An operation accordingly was carried out on April 8th under gas oxygen and ether anaesthesia. Convalescence was uneventful and a few days later the patient was about to be discharged from the hospital when she suddenly collapsed, became unconscious, cyanotic and dyspnoeic. It was recognized that she probably had a pulmonary thrombosis and an emergency operation was carried out with the removal of a small embolus from the right branch of the pulmonary artery. This operation failed to accomplish its purpose.

The autopsy confirmed the clinical diagnosis of arteriosclerotic heart disease, endometrial polyp, and acute pulmonary embolism. In addition there was found a duodenal ulcer, a slightly enlarged heart, slight atheroma of the coronary arteries without constriction, and a moderate atheroma of the aorta.

The *pituitary body*, which was found to occupy a definitely enlarged sella, was small and flattened. It shows (Figs. 26 and 28) a massive basophilic invasion from the pars intermedia which is

visible to the naked eye. Everywhere between the viable cells the holocrine product is well preserved (Fig. 27), the shadows of the swollen nuclei being still discernible in many of the cast-off cytoplasmic masses.

Sections from a gland showing a degree of posterior lobe infiltration perhaps even more marked than in the preceding example have been kindly sent to me by Dr. John F. Noble, through the intermediation of Professor Rasmussen. The history of the case is as follows.

CASE 13. The patient, 90 years of age, was admitted to the Ancker Hospital of St. Paul on Jan. 13, 1933. Her past health had always been good and in her active years she had been the mother of twelve children. She was extremely obese and had a blood pressure of 176/110. She showed evidence of mental deterioration with marked excitation. Albumin was occasionally but not invariably present in the urine with a few hyaline casts. She died suddenly on March 16, 1933, supposedly from a coronary occlusion.

Postmortem examination showed excessive obesity, hypertrophy of the right heart, generalized atherosclerosis, and a terminal pulmonary thrombosis with marked oedema and congestion of the lungs.

The *pituitary body*, cut sagittally, proves to be a cup-shaped gland (Fig. 29) with a massive basophilic invasion occupying practically the entire anterior half of the pars nervosa (Fig. 30). The invading cells bud off in characteristic fashion from the vascular stalks (Figs. 31 and 32). These stalks show a larger amount of perivascular connective tissue than is usual and this may conceivably represent the consequences of a long-standing process with fluctuation in activity.

DISCUSSION

In venturing to interpret the posterior lobe basophilia of eclampsia, of essential hypertension, and of the atheroscleroses and nephropathies of the aged in terms of neurohypophysial activation, questions immediately arise which some attempt must be made to answer. How often does the process occur in persons of supposedly normal health? What, if any, is the relation of these basophilic elements of the pars nervosa to the cells of the pars distalis which appear to be identical in their tinctorial reactions and morphology? Are the invading basophils the source of all the recognized activities of extracts derived from the posterior lobe?

1. *The Frequency of the Process:* Doubtless some measure of posterior lobe activity is constantly maintained. And if, as is assumed, the pars intermedia is responsible for it and the number of free basophils is an indication of its degree, few glands would, if serially cut, fail to show here and there an occasioned ripened cell wandering into the pars nervosa. But how often there occurs a massive invasion, as in some of the cases that have been cited, is impossible to say for want of routine postmortem studies of the gland with this particular point in view.

Only a few writers on the pathology of the hypophysis specifically mention these "inwandering" elements. Those who do, like Kiyono²⁵ (1926), merely allude to the fact without interpretation. Nor could there scarcely be any, for in his brief protocols of fifty-three cases thirty-two showed no invasion, twelve a slight invasion, and only nine a copious invasion. In this last group, four were examples of vascular disease, three of carcinoma of the breast, one had a brain tumor, and the ninth (the only subject below middle age) was a suicide. Rasmussen, in his valuable paper³⁸ (1930) dealing with the pars intermedia, depicts a single example of heavy infiltration without commenting on its possible significance. In a personal communication he states that in his collection of 240 serially cut glands a corresponding degree of invasion has been observed only half a dozen times.

The late Dr. Ernest Southard, for a number of years when pathologist to the Department of Mental Diseases, methodically collected and sectioned the pituitary bodies of the patients who had died in the Massachusetts state hospitals. His successor, Dr. Canavan, who continued to add to the material, has kindly permitted my co-worker, Dr. Eisenhardt, to go through these sections to get a general idea of what they show. The glands were uniformly cut through the middle on the horizontal plane so the single sections of each that have been preserved are apt to transect the outer angles of the posterior lobe, where the cellular invasion in question is most likely to be seen.

Unfortunately the case histories that go with the specimens are brief. They chiefly relate to the mental status of the patients, and when factors such as blood pressure are mentioned it is difficult to tell when the reading may have been taken, for many of the patients had long been inmates of the institutions in which they died. But

leaving all else aside, in a series of 100 of these glands, 64 per cent showed no basophilic invasion of the pars nervosa whatsoever, 23 per cent showed a slight invasion, and 13 per cent showed a marked invasion. The average age of the thirteen cases was 56 years, the ages ranging from 34 to 83. In only two instances was the age below 40: one was a man of 34 who died of lobar pneumonia, the other a woman of 38 with a blood pressure of 170/90 and a postencephalitic syndrome. The conditions in the other cases were so variable as to baffle analysis. Naturally many of the older patients were found at autopsy to have had arteriovascular disease.*

As a check on this series of Dr. Southard's, sections from a large collection of pituitary bodies made at the Johns Hopkins Hospital many years ago have been gone over, those in which the posterior lobe does not happen to be well shown having been excluded. A consecutive series of 100 of these sections from different glands shows in sixty-two no basophilic infiltration, in twenty-two a few invading elements, in nine a moderately well marked invasion, and in only seven a heavy invasion. Serial sections would of course have increased the number of positive cases. As matters stand the percentages in this and in the Southard series are surprisingly close.

Much depends naturally on what the terms "slight," "moderate" and "heavy" indicate, and without suitable illustrations different writers might have different views on the matter. However this may be, it may be gathered that in the general run of autopsies a heavy posterior lobe invasion is not infrequent. For though Rasmussen's estimate is low, namely, 2.5 per cent, my series showed 7 per cent and the Southard series 13 per cent, while in Kiyono's smaller group of cases 17 per cent showed marked invasion.

2. *The Function of the Pars Intermedia*: In accordance with the view that the pars intermedia must be the sole source of whatever active principle can be extracted from the posterior lobe, the pars nervosa is merely the carrier for the secretory product. This is assumed to find its way in the loose tissue toward the tuberal nuclei, and the broken-up appearance of the ependymal cuticle of the infundibulum¹² strongly suggests its partial passage into the cavity of the ventricle.

* In a small selected group of forty-two imbeciles and idiots, in whose study Dr. Southard was particularly interested, thirteen (or 31 per cent) showed marked invasion. The ages ranged from 24 to 72 years, many of the patients having been institutionalized for a long period of years.

Whether the secretion of the pars intermedia under variable stimuli is capable of being chemically altered, or whether its pharmacological action can be *qualitatively* modified during its transit through the nervous tissue, is now impossible to say. But there can be little doubt that under different physiological stresses or differing conditions of disease it is *quantitatively* variable, the degree of basophilia, as already indicated, being looked upon as a measure of posterior lobe activity.

Granted that a few invading basophils may normally be found in every gland that is completely studied, how rapidly their number may multiply under proper stimulation is unknown. Karplus and Peczenik,²⁴ to be sure, have shown that electrical excitation of the tuber will promptly increase the amount of a posterior lobe-like substance in the ventricular fluids. But whether such a stimulus long continued would actually lead to histological changes indicating activation of the pars intermedia does not appear to have been put to the test.

From Cannon's experiments it is known that the adrenal medulla may be quickly activated and there is no reason to believe that the response of the neurohypophysis to an electrical or emotional stimulation would be any less slow. In the case of the adrenal glands, however, we do not yet know just where to look microscopically for the cytological source of the pressor principle, whereas in the neurohypophysis we apparently now do.

The several pathologists whose opinion has been consulted in regard to these matters have mostly raised the objection that a posterior lobe basophilia may occasionally be encountered in supposedly normal glands. Professor W. G. MacCallum and Professor H. M. Turnbull have both sent me sections from the pituitary bodies of persons who have died in consequence of accidents, the glands showing (Figs. 33 and 34) as rich a basophilic invasion as was present in some of the cases of eclampsia herein described.

Just what form of neurohumoral stimulation calls forth the basophilic invasion in the first place is undetermined. But it is known that the posterior lobe receives a richly arborized, non-myelinated nerve supply from the anterior hypothalamic nuclei and its functional activity is probably controlled by a diencephalic mechanism that is highly sensitive to the primitive emotions. And if, as Cannon has shown, the sympathico-adrenal apparatus can be discharged

by fright, there is every reason to suppose that the neurohypophysis is just as likely, if not more likely, to respond to crude stimuli of similar kind.

That the *pars intermedia* cells, under profound or prolonged nervous impulses, can multiply and ripen with sufficient rapidity to invade the lobe and discharge their secretion so as to produce in the course of a few hours the pathological picture under discussion may be assuming too much. Granting that there was no preëxisting hypertension of which the postmortem examinations gave no evidence, and being unaware of how long the patients survived, this is the only possible present explanation to offer for the basophilic infiltration of the posterior lobe in these fatal accident cases. However this may be, and some better explanation may be forthcoming, it is the purpose of this paper to offer an interpretation of those instances of posterior lobe basophilia that are associated with a *known* disorder, rather than to attempt an explanation for all conditions in which a similar process is found to occur.

3. *Posterior Lobe Secretion and the Invading Elements*: What are these basophilic elements that are taken to be activators of the posterior lobe, and what is their relation to the basophilic cells of the *pars distalis*? From the fact that in the case of pituitary basophilism not only was there a basophilic adenoma of the *pars distalis* but at the same time a marked invasion of the posterior lobe, it might be assumed that the elements in both regions had been simultaneously affected by the same stimulus, whatever it might be. A wholly similar dual basophilia affecting both lobes has also characterized some of the eclamptic glands that have been studied. Histological similarity, however, does not necessarily imply that the chemical nature of the secretory product of the cells is identical. While loth to get entangled in the highly controversial subject of the relation of the anterior pituitary-like substance, *prolan*, to the actual gonadotropic hormone of the anterior hypophysis, something nevertheless must be said regarding it in connection with the subject in hand.

Emphasis up to this point has been laid on hypertension as a manifestation of the posterior lobe activation, rather than on other less striking and less easily measurable symptoms, but this does not mean that other effects, such for example as disturbances of carbohydrate and fat metabolism, which are equally well ascribable to posterior lobe over-activity, may not at the same time be produced. The associa-

tion of diabetes mellitus with adiposity and subsequent hypertension has long been appreciated in the clinic and the suspicion of a concomitant (possibly primary) pituitary disorder been aroused. That all three of these conditions are striking features of pituitary basophilism can scarcely fail to be of significance.

That the posterior lobe might contain a gonadotropic substance, however, would scarcely be expected. Pighini ³⁵ (1932) has reported that extracts of the human anterior hypophysis and tuber, as well as the cerebrospinal fluid from the third ventricle, give positive Aschheim-Zondek tests in immature rats. There would, however, be no way of telling whether the gonadotropic substance in tuber and cerebrospinal fluid had been transported from pars distalis by the hypophysio-portal veins or whether it had come from the pars intermedia. To this question with great profit Zondek and his collaborators have recently turned their attention.

One of the well known properties of posterior lobe extracts obtained from the glands of animals is its melanophore-expanding capacity when tested on batrachians. While the posterior lobe hormone or hormones are not normally present in sufficient amounts in the blood to be definitely detectable it had, however, been observed by Küstner, by Ehrhardt, by Dietel and others, that a melanophore-expanding substance appears in the blood serum of pregnancy and can be found in high concentration in the serum of eclamptics.

Zondek and Krohn ⁴⁸ a year ago (1932), after a series of ingenious experiments in which the European minnow was used as a highly satisfactory test object for the melanophore reaction, announced that the juxtaneural strip of both the human and bovine hypophysis contains an excess of this component of posterior lobe extracts which is neither detectable in the pars distalis nor in the remote portions of the pars nervosa. The active substance, which was called "intermedin," can be traced through the stalk and the tuber, and it is demonstrable in small amounts in the fluid content of the third ventricle, though not elsewhere in the cerebrospinal fluid spaces.

Thus one at least of the constituent properties of posterior lobe extracts has been shown to be more highly concentrated in the zone of the pars intermedia from which it is in all certainty elaborated. But Zondek has gone still further and in the present year (1933) has shown ⁴⁷ that in the human (but not in the bovine) hypophysis a sex-

maturing substance identical with prolan A is present in this same strip of posterior lobe which lies adjacent to its epithelial investment. Traces of it are also found in the stalk but not in the third ventricle, in which respect it differs from intermedin. Under the influence of Berblinger, Zondek concludes that this substance represents the in-wandering basophils from the pars distalis (*sic*). Prolan, he believes, must therefore be derived from the basophilic elements of the anterior lobe.

From what source the human glands used in these experiments by Zondek were obtained and to what maladies the subjects may have succumbed is not mentioned. Nor could the tissues have been used both for the making of an extract and for the histological demonstration or otherwise of an active posterior lobe basophilia. It is quite probable, however, that had the posterior lobe activation by basophils in these glands been sufficiently marked, the sex-maturing substance might also have been demonstrable in the fluid of the third ventricle. Hints suggesting this possibility have been provided from another source, namely, from the studies by certain gynaecologists. The evidence at hand has been summarized briefly as follows by Eugen Kulka.²⁹

Aschheim, in searching for follicle-ripening substances in various fluids and tissues of pregnant women, failed to find any trace of such a substance in the cerebrospinal fluid. Califonza, on the other hand, believed that he had detected its presence in fifteen out of the twenty-eight fluids examined. Ehrhardt¹⁶ found prolan A in the cerebrospinal fluid in three cases of eclampsia, in one preëclamptic, and in a gravid woman suffering from carcinoma; and Heim states briefly that he had corroborated these findings in eclamptics. Kulka investigated the lumbar fluid from twenty-five gravid patients, seven of them with symptoms of marked eclampsia. The Aschheim-Zondek test was negative in the fluid in all but six of the patients. Of the six cases showing a positive reaction one had intra partum eclampsia with a blood pressure of 190, labour having been induced by forceps. Another was a postpartum eclamptic with a blood pressure of 200, oedema of the extremities and albumin in the urine. The third patient had a cystic chorionepithelioma and three others were examples of marked hyperemesis gravidarum.

While the evidence given by these several writers is suggestive rather than conclusive, it is remarkable that under any circum-

stances of posterior lobe activation an active principle should be found in the cerebrospinal fluid obtained by lumbar puncture. Could the fluid from the ventricles have been examined, or even that from the posterior cistern, the chance of detecting the substance looked for would have been vastly greater.

More important are the recent biochemical studies by Anselmino and his collaborators, to which allusion has already been made. In their more recent paper² (1932) it is claimed that the active substances found in the blood of eclamptics are identical with the corresponding fractions of posterior lobe extract and that their amount varies quantitatively with the severity of the symptoms. They assume that the combination of excessive pressor and antidiuretic effects leads to arteriolar and capillary spasm with water retention and oedema of the tissues. When the brain becomes oedematous convulsions and coma are produced and there is usually a terminal oedema of the lungs. They believe that overproduction of the posterior pituitary hormone affords the only consistent explanation of these phenomena. All this seems the more plausible in view of certain observations such as those by Rowntree,³⁹ by Dietel,¹⁵ and by McQuarrie and Peeler³² on the clinical and pathological consequences of experimental water intoxication, whether produced by administering excessive amounts of water or by the antidiuretic effect of posterior pituitary extracts.*

While the studies mentioned above are highly suggestive, they are concerned with some of the better known properties of posterior lobe extracts and have no apparent bearing on the possible production by the posterior lobe of the sex-maturing substance that Zondek has found to be present in the juxtaneural portion of human glands. In this connection the following observations would seem to be of great significance.

Drs. G. Van S. and O. W. Smith of Boston have recently shown⁴¹ that the blood and urine of toxæmic patients in late pregnancy contain a far larger amount of the anterior pituitary-like substance (prolan) than ordinarily occurs in pregnancy. They have further demonstrated⁴¹ in a second communication that a quantitative imbalance between prolan and oestrin is characteristic of the

* Efforts to produce in animals lesions in the liver and kidneys comparable to those characterizing human eclampsia by administering posterior lobe extracts have been highly contradictory (*e. g.*, the papers by Dietel,¹⁵ by Fauvet,¹⁶ and by Ohligmacher⁴⁹).

toxaemias of late pregnancy. The number of rat units per 100 cc. of blood serum in twelve gravid women without symptoms averaged 50, in eighteen toxæmic patients 250, and in five eclamptics 480. The amount of oestrin was correspondingly diminished. In the course of this study the interesting observation was made on a gravid woman with diabetes insipidus that the amount of pituitrin necessary to control the polyuria was greatly diminished during the months of child-bearing.

It can be gathered from all this that information from many sources points toward a hyperactivation of the posterior lobe in these hypertensive states. And if we are to believe, as some of the observations strongly suggest, that prolan is a product of posterior lobe basophilia, while the gonadotropic substance extracted from the anterior lobe is derived from the basophils of that part of the gland, the difference in the reactions of these two sex-maturing substances, which so many have pointed out, may thus be accounted for.

SUMMARY AND CONCLUSIONS

The active principle of the posterior lobe and its several fractions must under all circumstances primarily be derived not from the pars nervosa but from its epithelial investment — the pars intermedia.

When the posterior lobe of man is functionally dormant the pars intermedia is inconspicuous, but so soon as it is activated the investing cells become transformed into basophilic elements, which in certain areas invade the pars nervosa. When their cytoplasm becomes fully ripened the cells eventually lose their staining qualities, change first into discernible "hyaline bodies" and then into a fluid product, which apparently makes its way through the loose tissue spaces of stalk and tuber in the direction of the infundibular ventricle and the adjacent hypothalamic nuclei.

Under certain circumstances the invading basophils with their desquamated products are greatly increased in number and the cellular infiltration assumes a massive character. This is looked upon merely as a pathological exaggeration of the normal secretory process and its degree is regarded as a measure of the hyperactivation.

An extreme example of posterior lobe basophilia of this sort has been observed in a case of so-called "pituitary basophilism," associated with a functionally active basophilic adenoma of the pars distalis. This polyglandular disorder chiefly affects young persons and

is characterized, among other symptoms, by vascular hypertension together with disturbances of carbohydrate and fat metabolism. As these symptoms suggest a posterior rather than an anterior lobe effect, it was assumed that the posterior lobe basophilia represented something more than an overflow of these elements from the anterior lobe.

It has been shown by Anselmino and his collaborators that the blood of eclamptics with oedema and marked hypertension contains antidiuretic and pressor substances, whose effects correspond to those produced by posterior lobe extracts. They therefore claim to have proved what others had suggested, that the toxæmias of pregnancy were due to the overproduction of the posterior lobe hormones.

In serial sections of six out of nine pituitary bodies from fatal cases of eclampsia a heavy infiltration of basophilic elements in the posterior lobe has been disclosed, and the same condition has been observed in a number of glands from cases of essential or nephrovascular hypertension, also serially cut and examined. That in advancing years there is a tendency for the basophilic cells thus to wander in large numbers into the posterior lobe has long been known. It has been looked upon merely as a concomitant of old age, particularly when attended by atherosclerosis and renal disease.

Pathologists have recognized in eclampsia distinctive lesions in the liver to which the disorder has customarily been ascribed. In essential hypertension, likewise, lesions affecting the terminal arterioles of the kidneys have been thought to indicate a primary nephrovascular disorder. Necroses in eclampsia, however, are not limited to the liver, nor are the terminal arteriolar lesions in essential hypertension confined to the kidneys. In neither instance do the histopathological findings satisfactorily account for the clinical symptoms.

From the observations presented the conclusions are drawn: (1) that the source of these hypertensive disorders lies in the posterior lobe of the pituitary body; (2) that the extent of basophilic invasion from the pars intermedia is a measure of posterior lobe activity; and (3) that excessive infiltration by these elements represents the histopathological basis of eclampsia and essential hypertension in young persons and may possibly also be related aetiologically to the atherosclerosis of old age.

Whether the general hypothesis herein advanced should or should not prove on further study to be in all its features wholly correct, it will nevertheless provide an incentive to include a detailed study of the neurohypophysis in forthcoming postmortem studies of disorders in which hypertension is a distinguishing feature.

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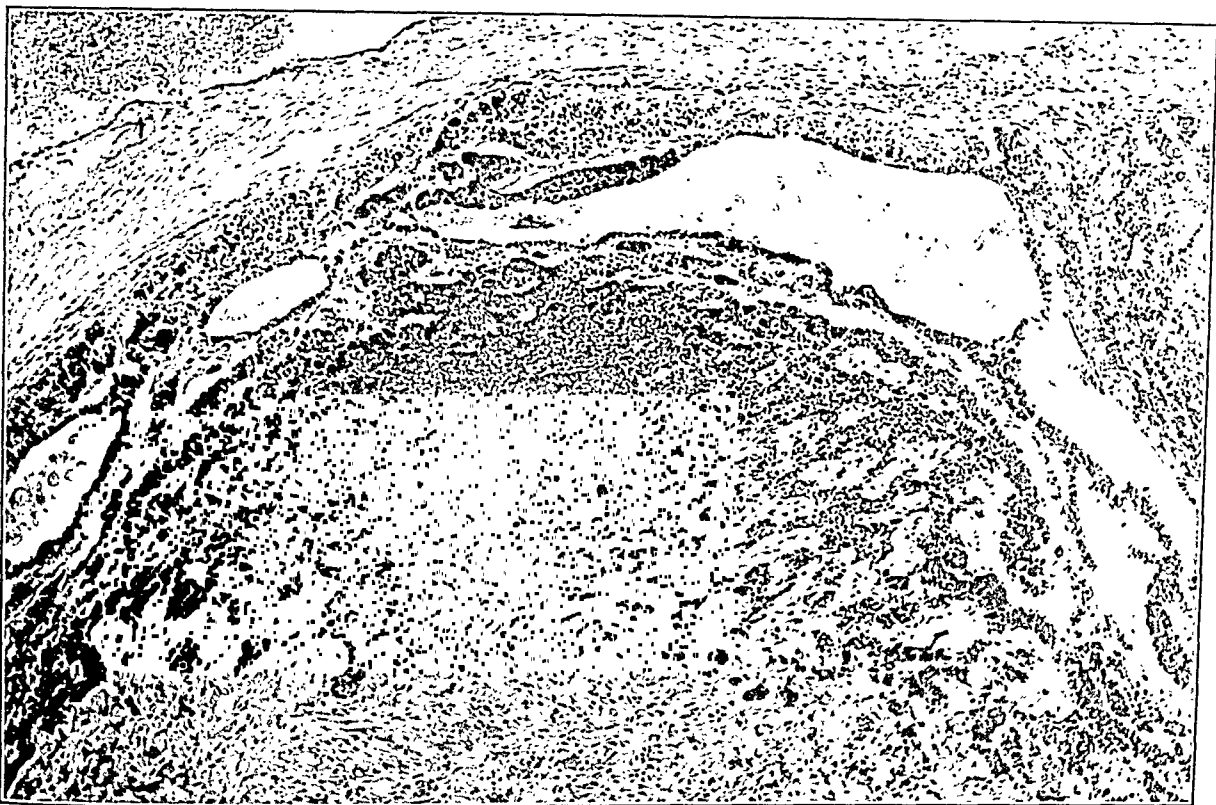
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DESCRIPTION OF PLATES

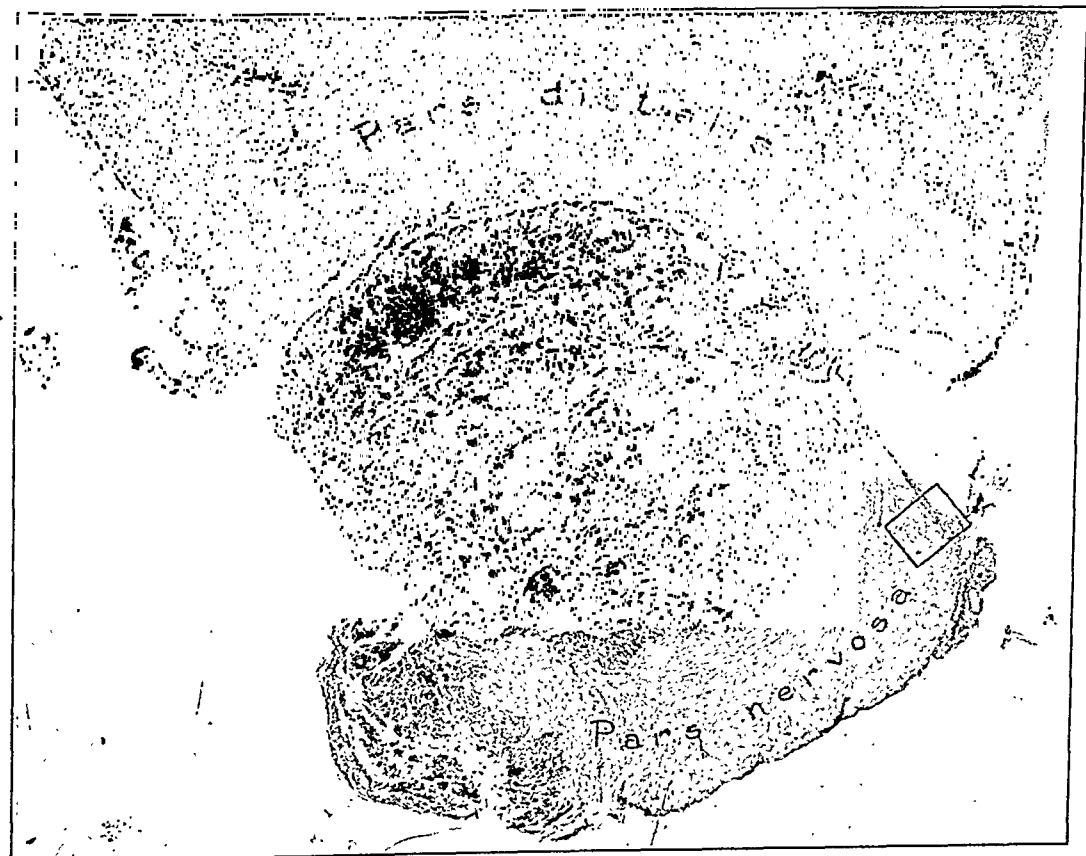
PLATE 54

FIG. 1. Massive posterior lobe invasion from a case of pituitary basophilism (mag. $\times 60$).

FIG. 2. Section (mag. $\times 9$) from the Raab-Kraus case of basophilic adenoma presumably arising from pars intermedia (*cf.* Fig. 3).



I



2

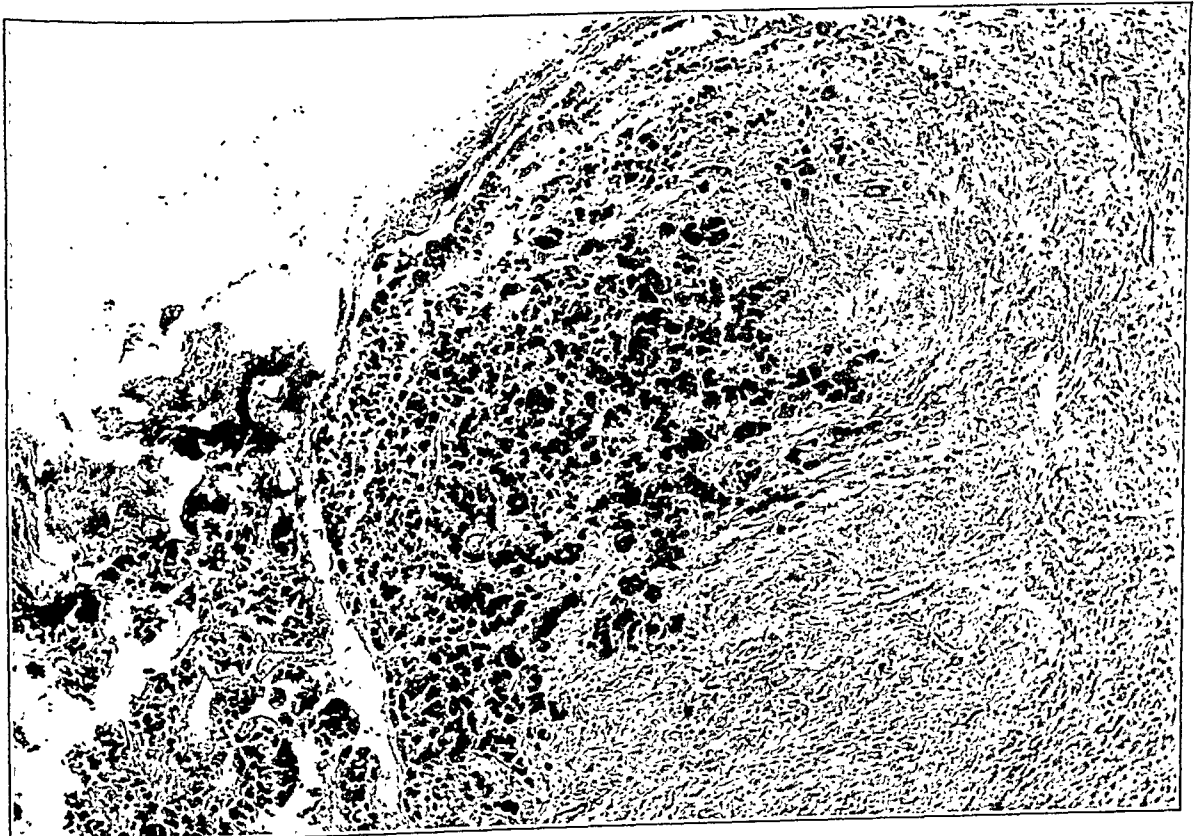
PLATE 55

FIG. 3. Squared area from Fig. 2 (mag. $\times 80$) showing infiltrating basophils in a zone remote from the adenoma.

FIG. 4. Typical cone-shaped area of basophilic invasion from outer angle of pars intermedia in a case of presumed eclampsia (mag. $\times 80$).



3

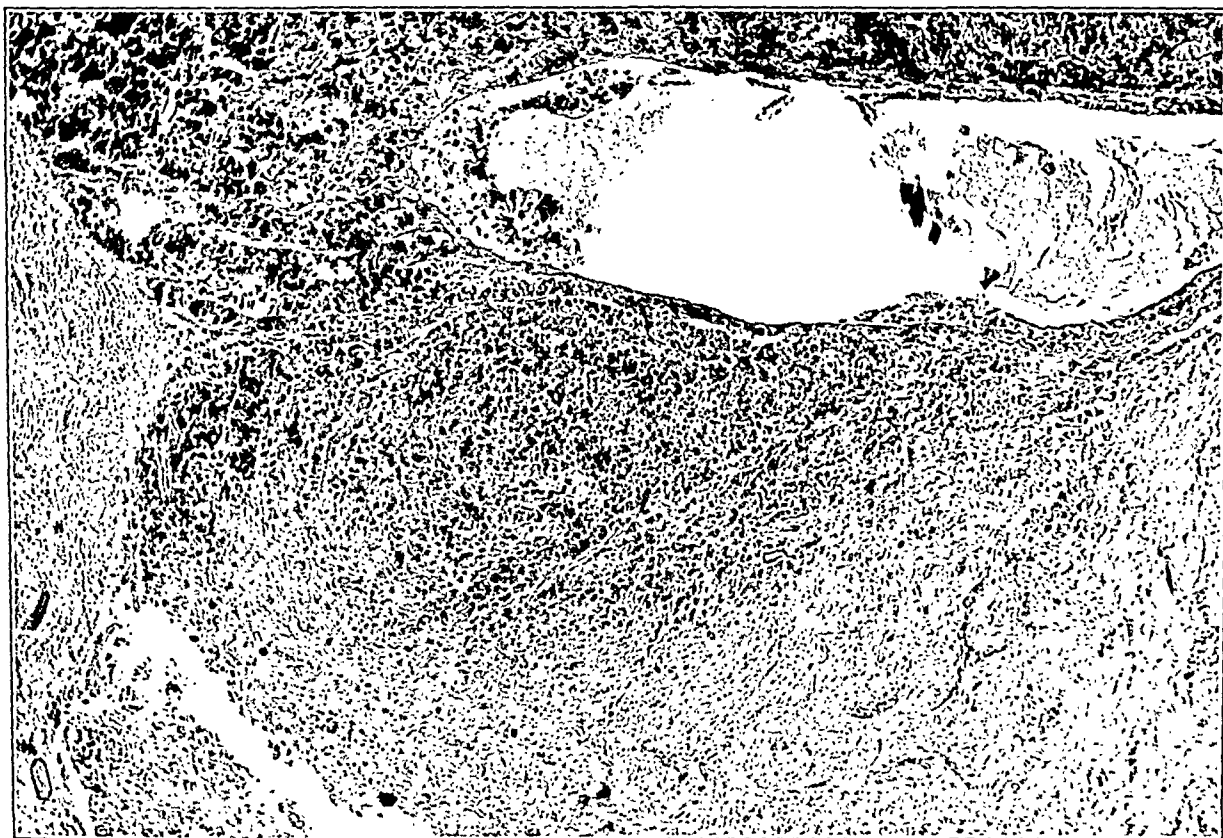


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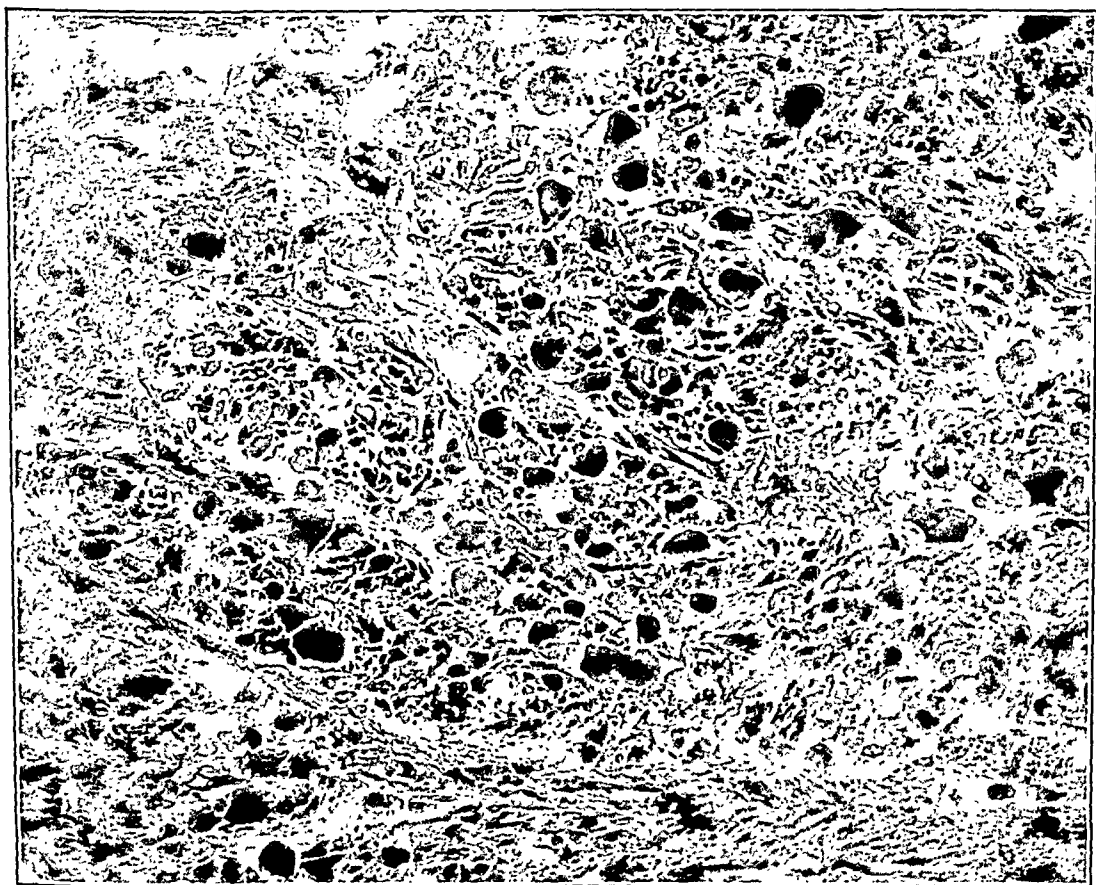
PLATE 56

FIG. 5. (Case 2.) Posterior lobe infiltration by basophils from a case of eclampsia with hypertension (mag. $\times 60$).

FIG. 6. (Case 2.) Showing (mag. $\times 300$) in center of pars nervosa accumulations of hyaline masses (Herring) in the spaces that are bounded by the "baskets" of neurofibrils.



5



6

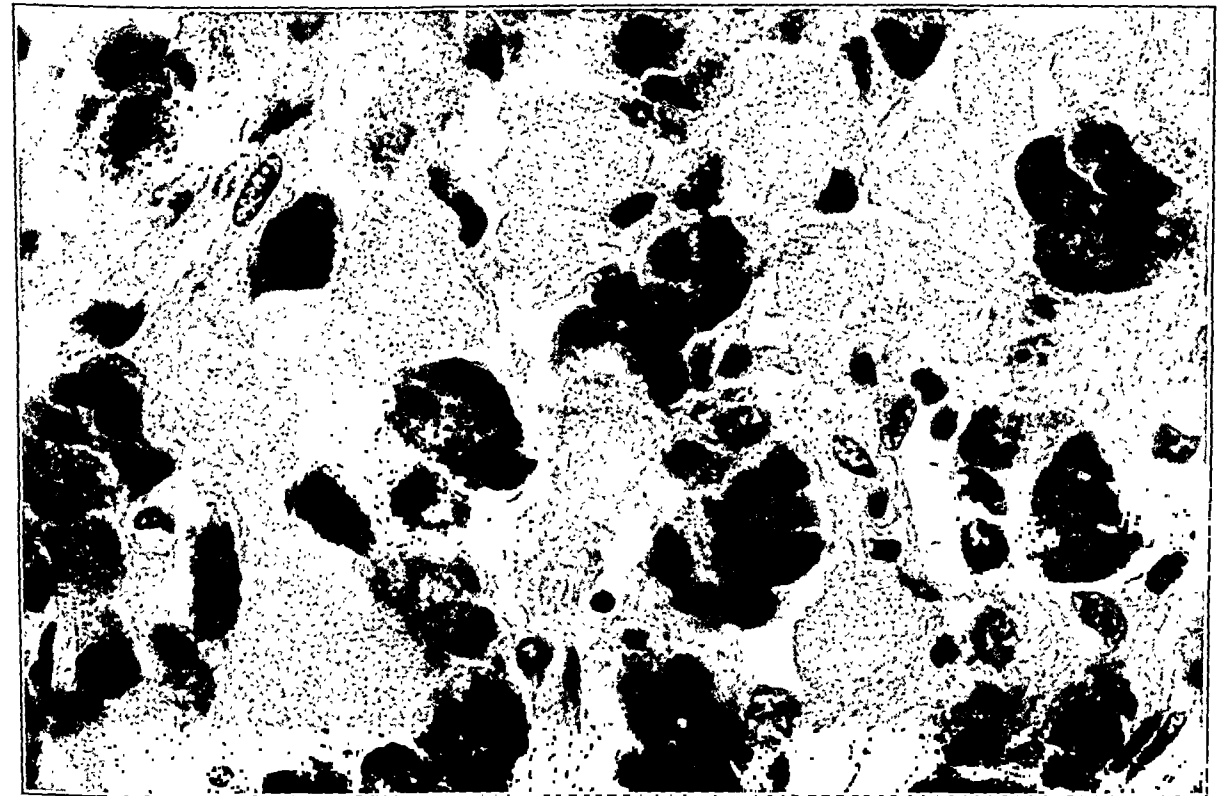
PLATE 57

FIG. 7. (Case 3.) Invasion of basophilic elements from pars intermedia in a case of eclampsia. Note separation from pars anterior (upper left) by large colloidal mass reopening residual cleft (mag. $\times 60$).

FIG. 8. (Case 3.) Showing (mag. $\times 560$) masses of granular holo-crine secretion in and among tongues of invading basophilic elements.



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Cushing

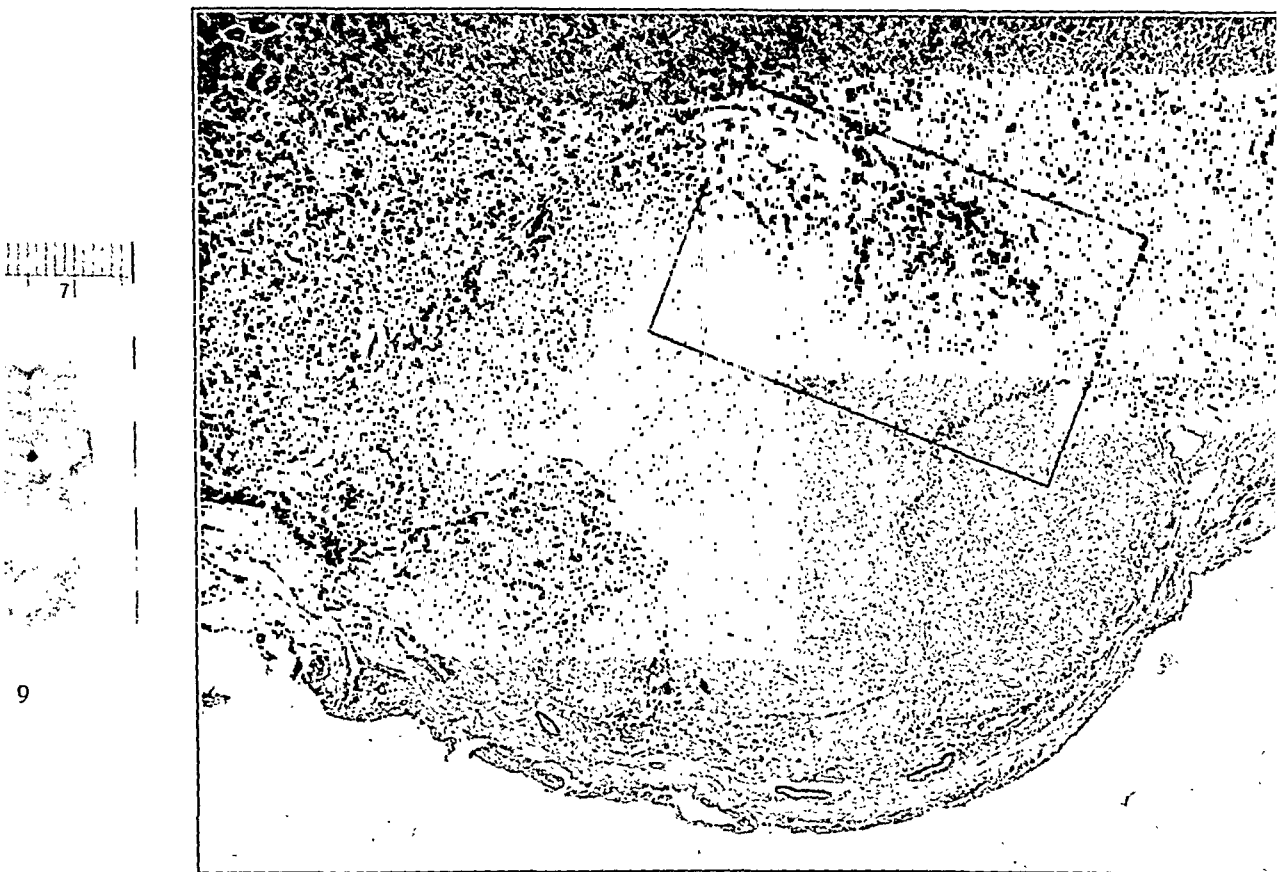
Hyperactivation of the Neurohypophysis

PLATE 58

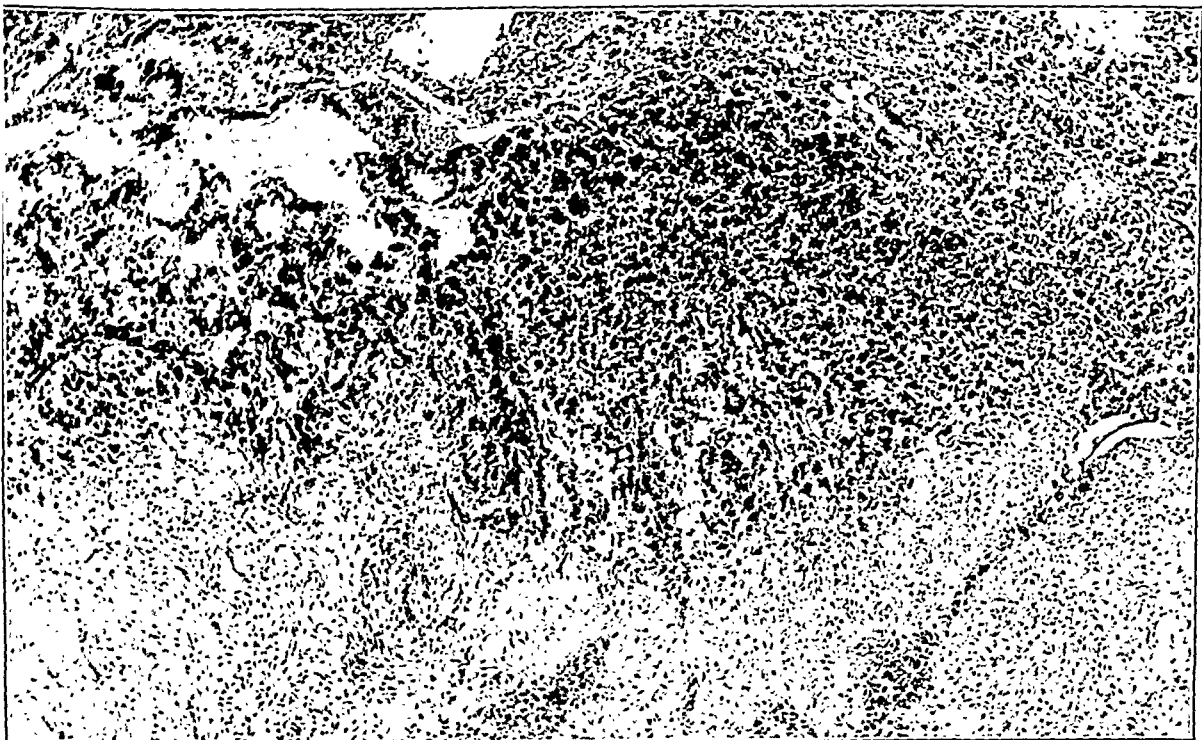
FIG. 9. (Case 4.) Posterior view of block of tissue with large gland, bulging posterior lobe and juicy tuber in case of eclampsia.

FIG. 10. (Case 4.) Horizontal section (Section 1260) through lower portion of posterior lobe to show widespread encirclement by actively invading basophils (mag. $\times 20$).

FIG. 11. (Case 4.) Showing area (mag. $\times 60$) squared in Fig. 10.



10



11

Cushing

Hyperactivation of the Neurohypophysis

PLATE 59

FIG. 12. (Case 4.) Showing (Section 3060, mag. $\times 15$) strand of basophils (arrow) in lower stalk. At this level through upper part of gland the pars tuberalis showing above has just become free from pars distalis. Insert (Fig. 12a) shows (Section 3510, mag. $\times 15$) same strand of viable cells still traceable in free stalk.

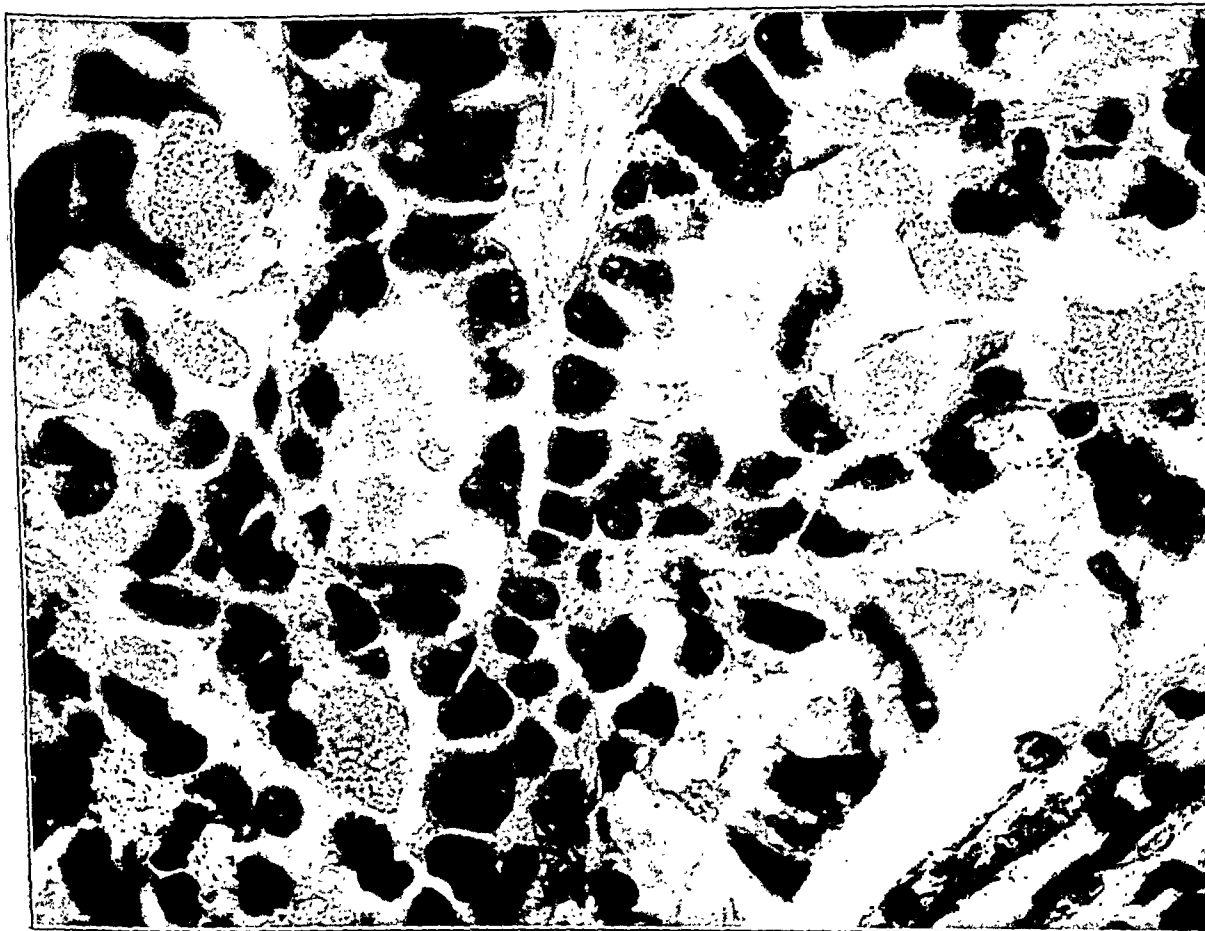
FIG. 13. (Case 4.) To show (mag. $\times 600$) the holocrine discharge of ripened cells between invading elements. Note ghosts of nuclei in several of the secretory masses.



12



12 a



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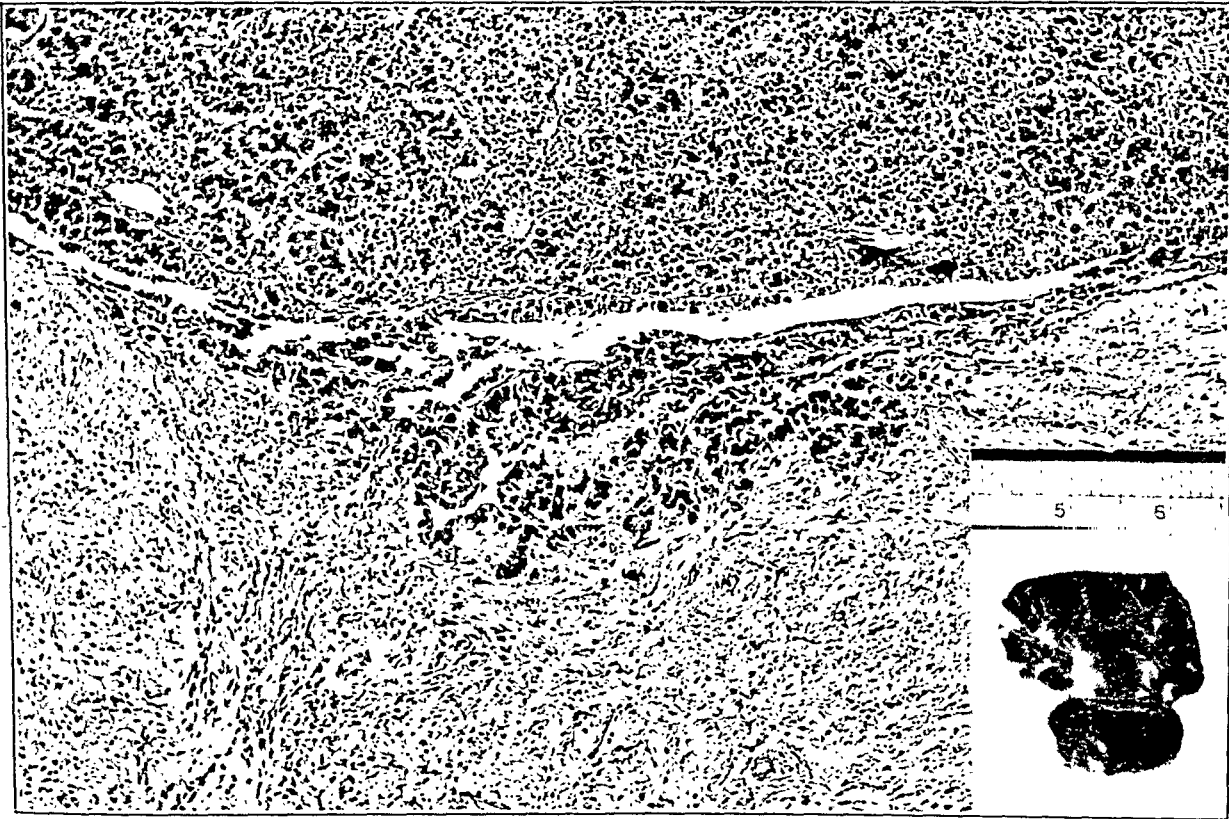
Cushing

Hyperactivation of the Neurohypophy

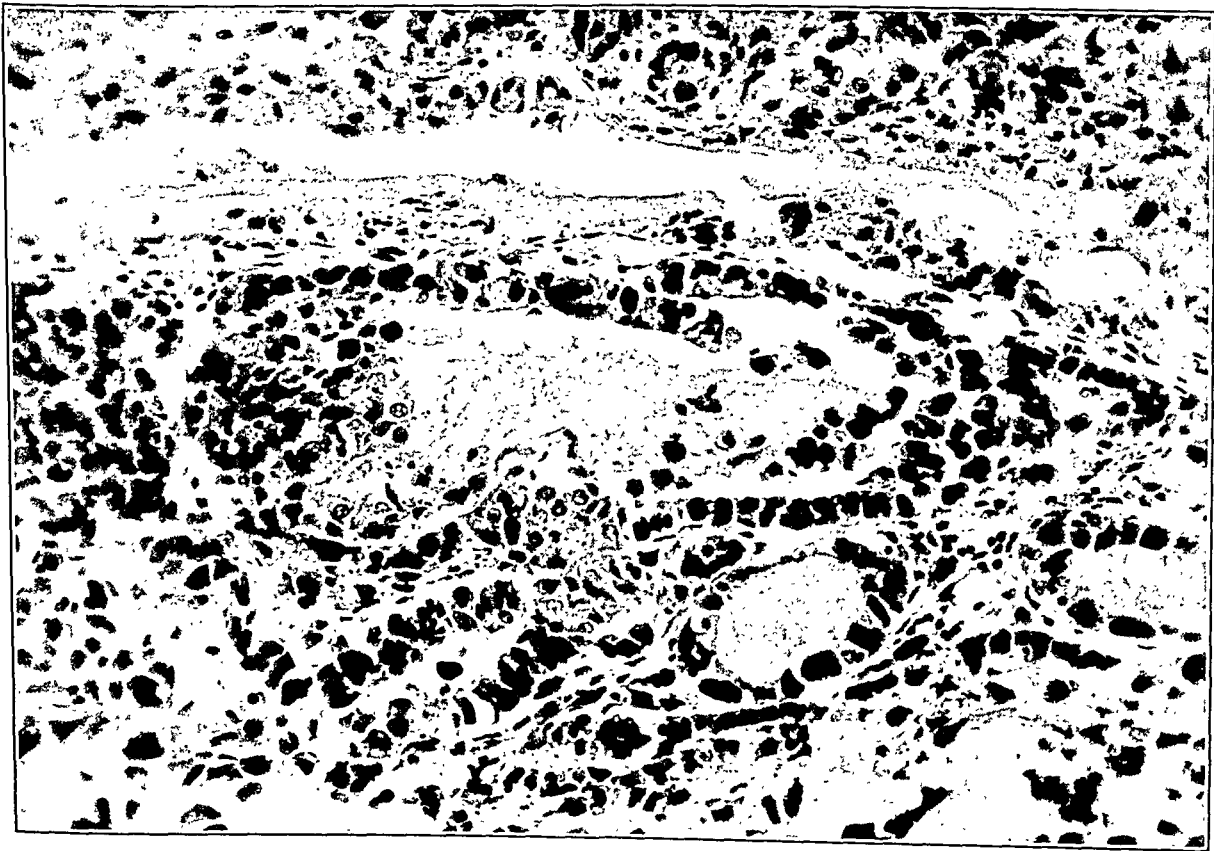
PLATE 60

FIG. 14. (Case 5.) Showing (mag. $\times 80$) one of two areas of moderate conical infiltration traceable in other sections into center of pars nervosa. Insert shows the posterior view of the specimen (natural size) with pituitary body below.

FIG. 15. (Case 5.) Showing (mag. $\times 230$) pars intermedia activity with formation of Rathke's cysts lined by ripened basophils. Pars distalis (above) separated by cleft from posterior lobe (below).



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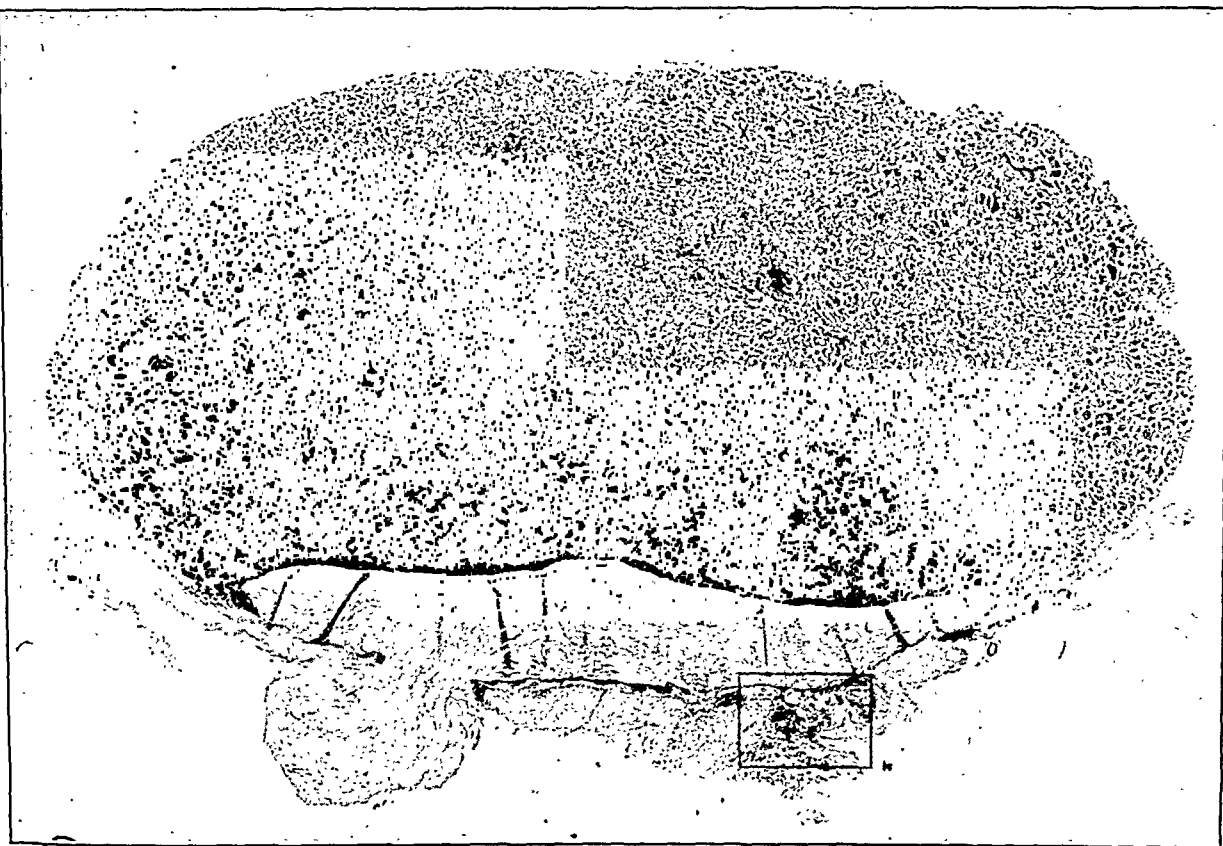
Cushing

Hyperactivation of the Neurohypophysis

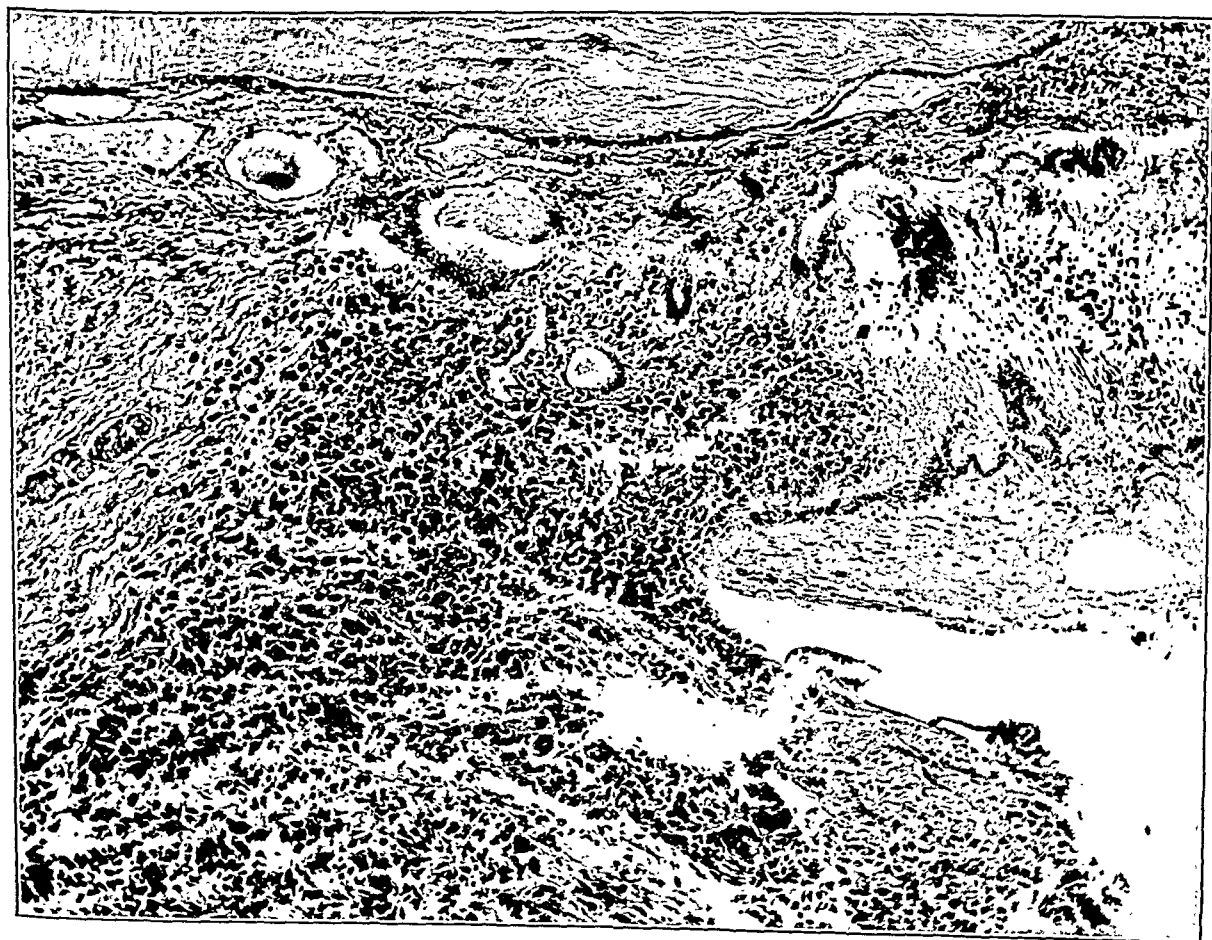
PLATE 61

FIG. 16. (Case 6.) Section 630 (mag. $\times 8$) showing large anterior lobe with entire cleft distended by colloid. Posterior lobe somewhat damaged in removal. Basophilic invasion from pars intermedia in squared area.

FIG. 17. (Case 6.) Squared area from above (mag. $\times 70$) showing invasion from pars intermedia.



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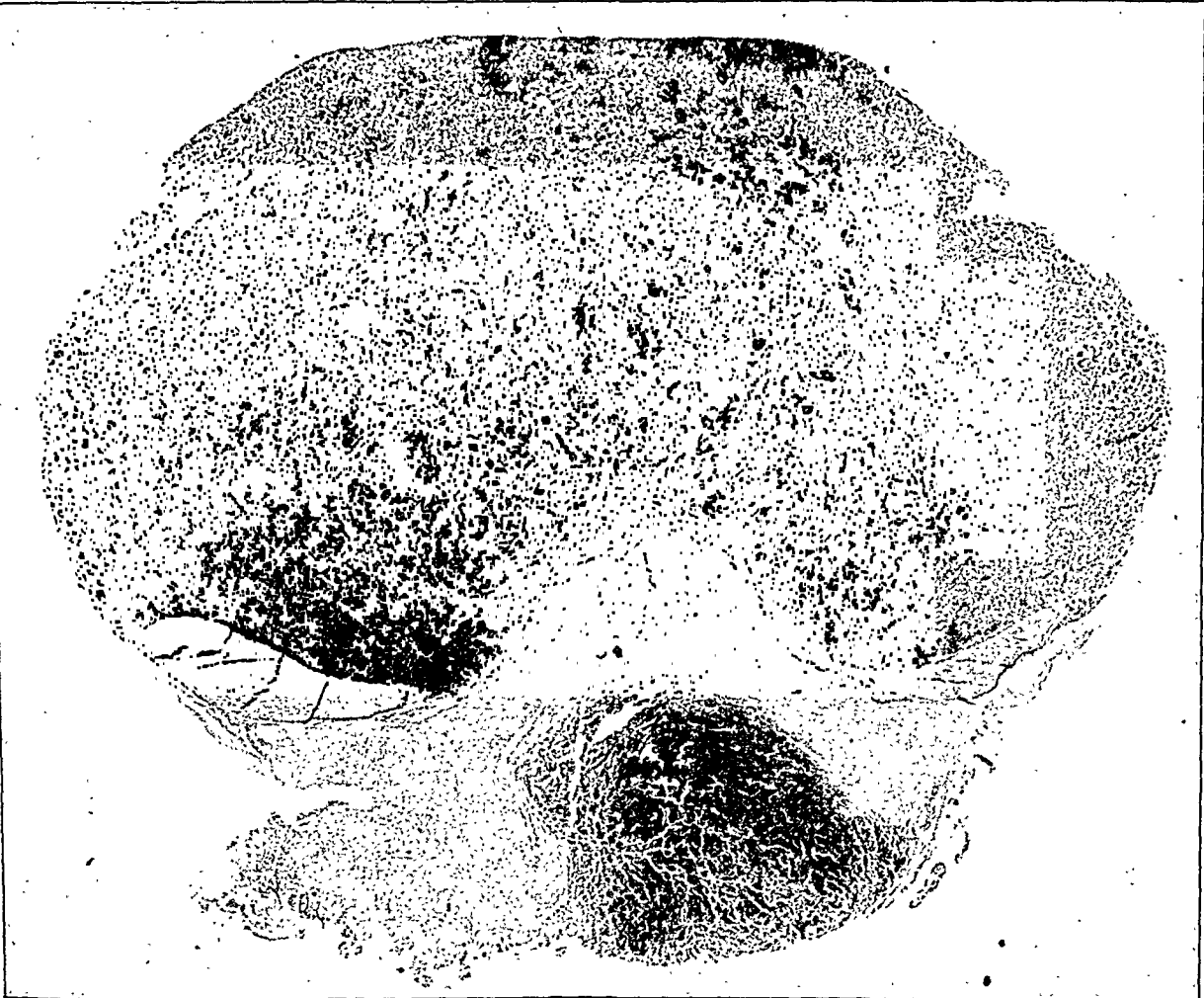
Cushing

Hyperactivation of the Neurohypophysis

PLATE 62

FIG. 18. (Case 6.) Section 1710 (mag. $\times 8$) taken at level where stalk of posterior lobe is forming and large portal sinusoids are congregating toward it. Note large basophilic adenoma in posterior lobe.

FIG. 19. (Case 6.) Section 2250 (mag. $\times 8$) showing adenoma fading off at posterior edge of pars nervosa. At this level the pituitary stalk has already formed and the portal vessels are clearly shown radiating backward toward it in what will become pars tuberalis.



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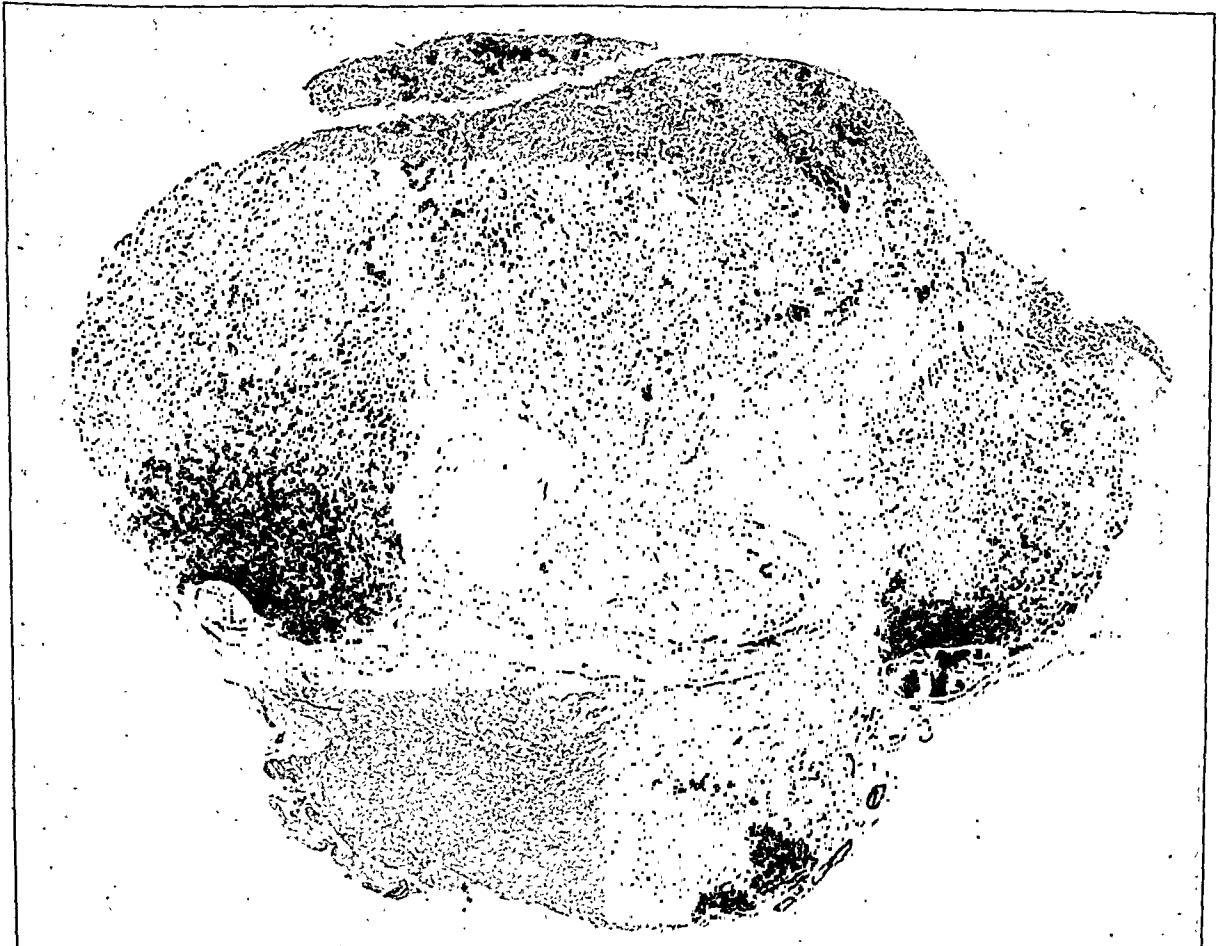
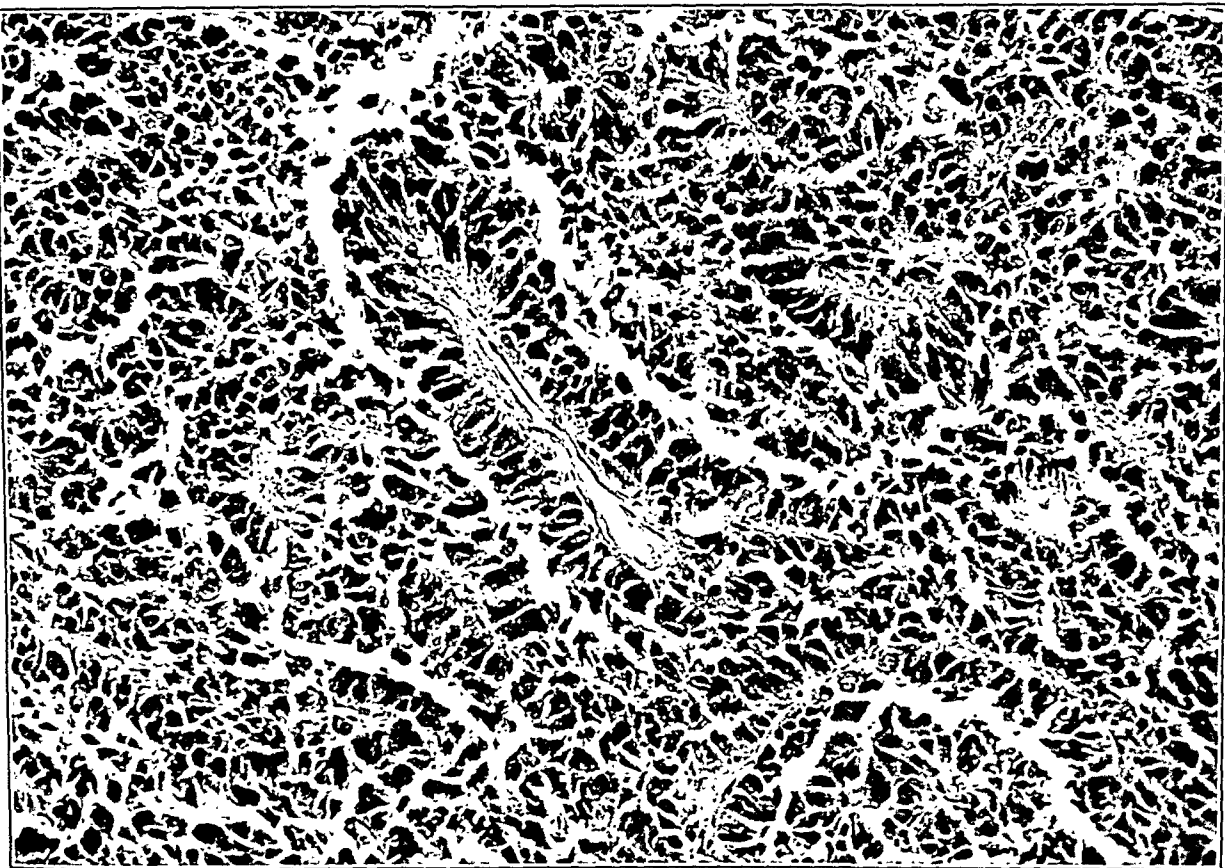


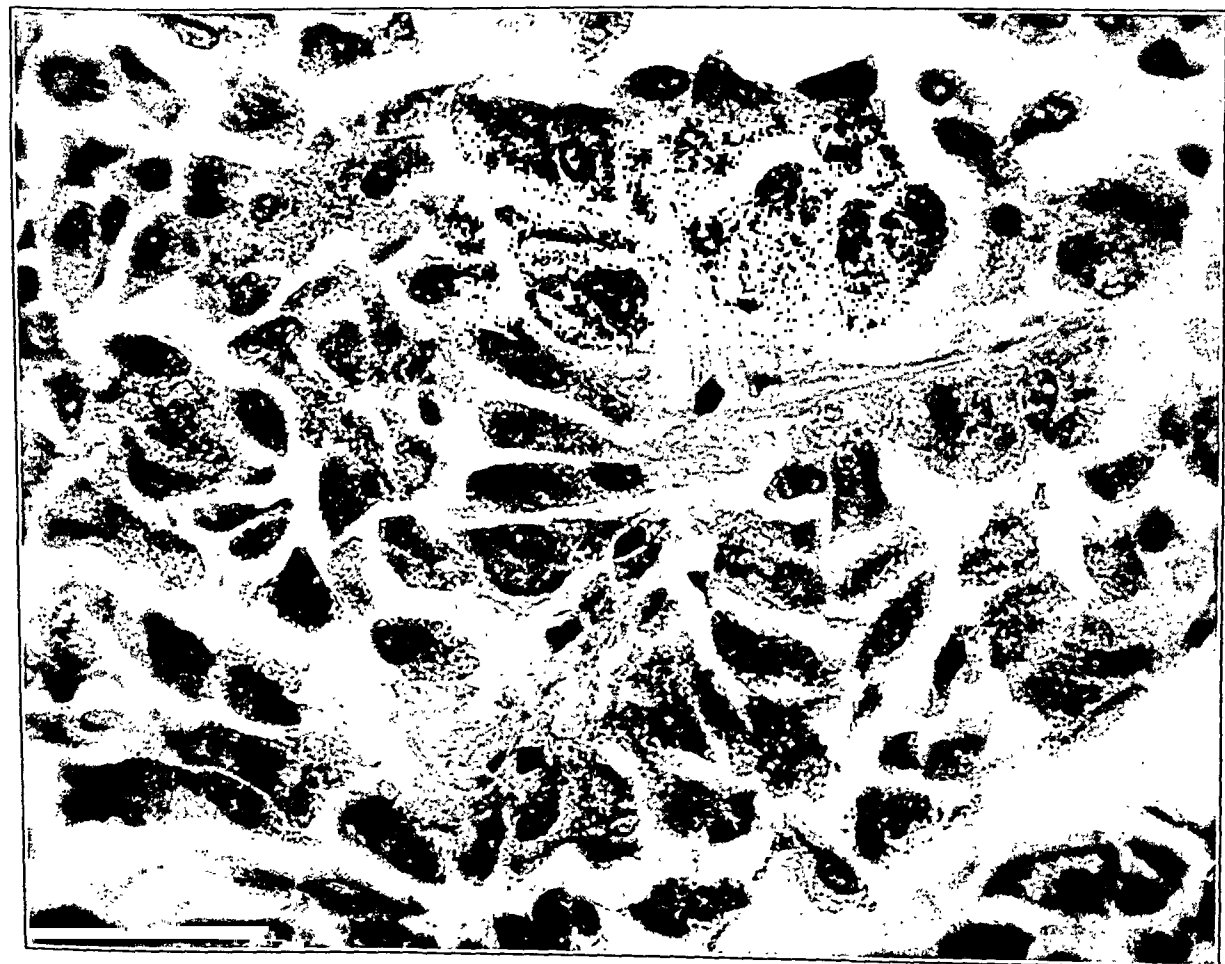
PLATE 63

FIG. 20. (Case 6.) To show (mag. $\times 150$) general character of adenoma whose cells bud off from capillary stalks.

FIG. 21. (Case 6.) Showing on higher magnification ($\times 600$) the typically vacuolated basophilic elements of the adenoma.



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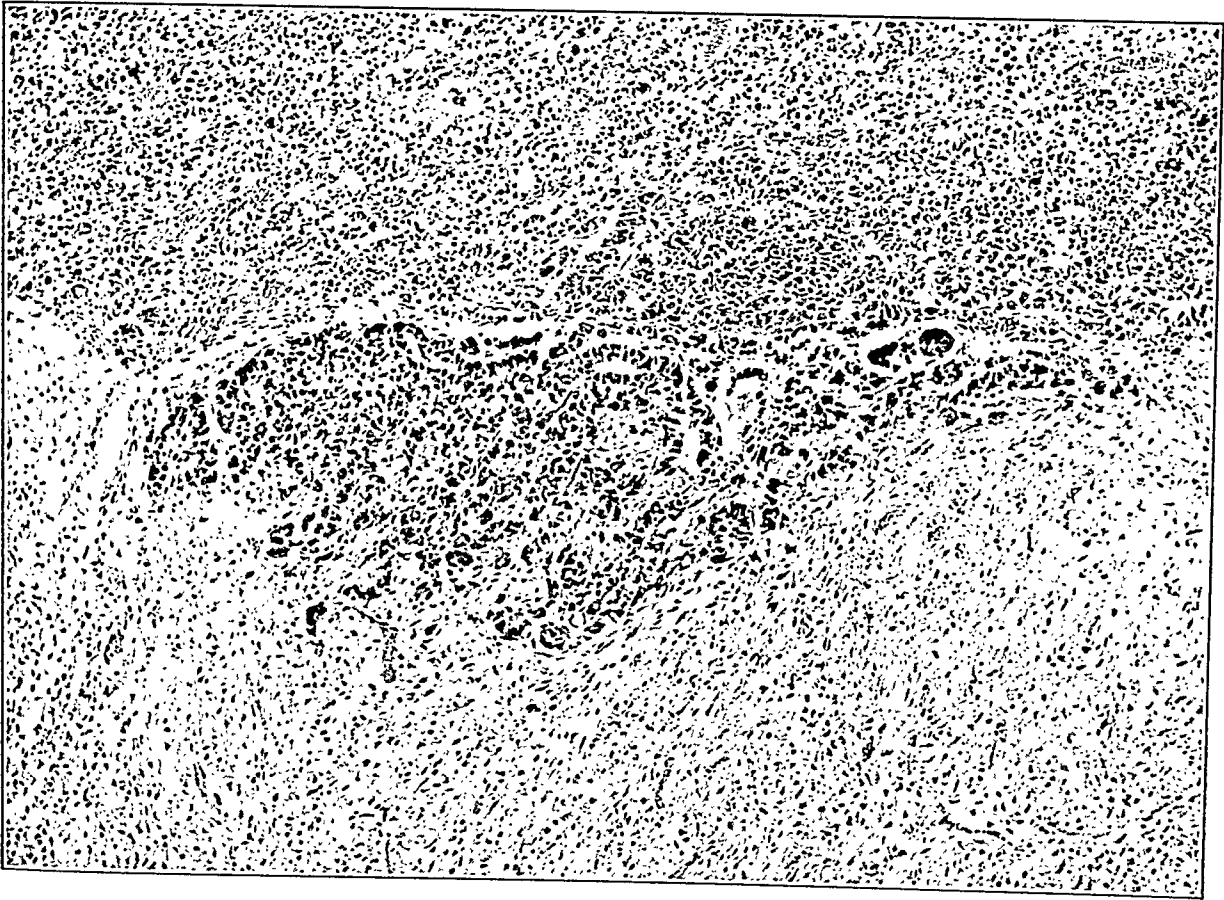
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Cushing

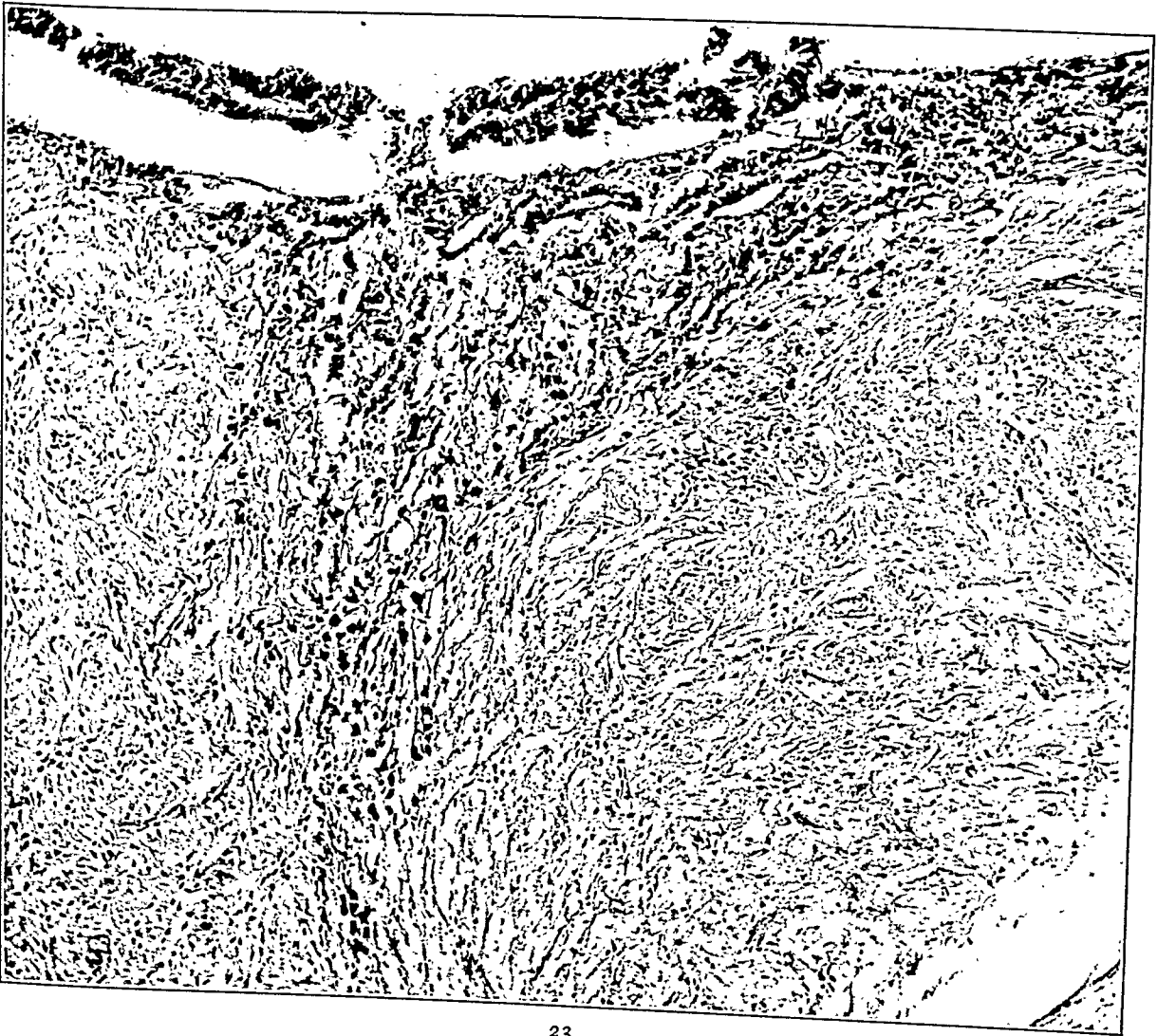
Hyperactivation of the Neurohypophysis

PLATE 64

FIGS. 22 and 23. Showing (mag. $\times 80$) the relatively slight degree of invasion in Case 7 (above) and Case 8 (below).



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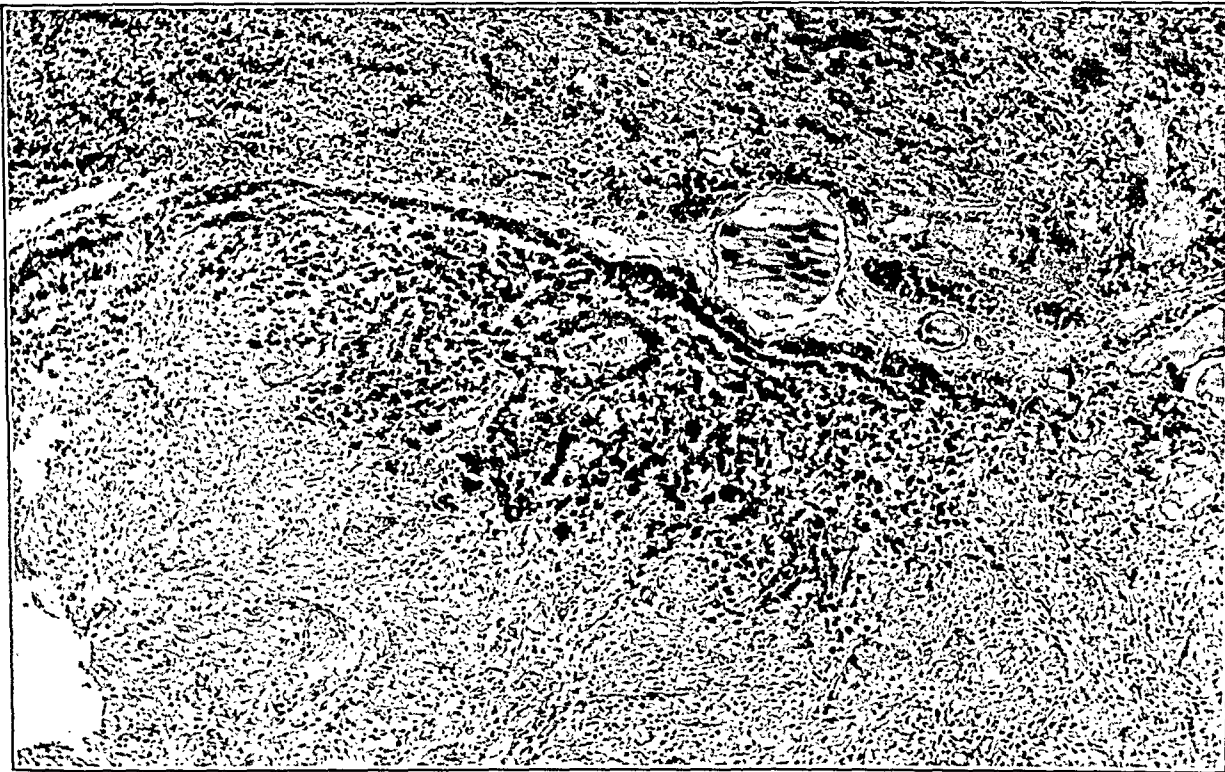
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Hyperactivation of the Neurohypophysis

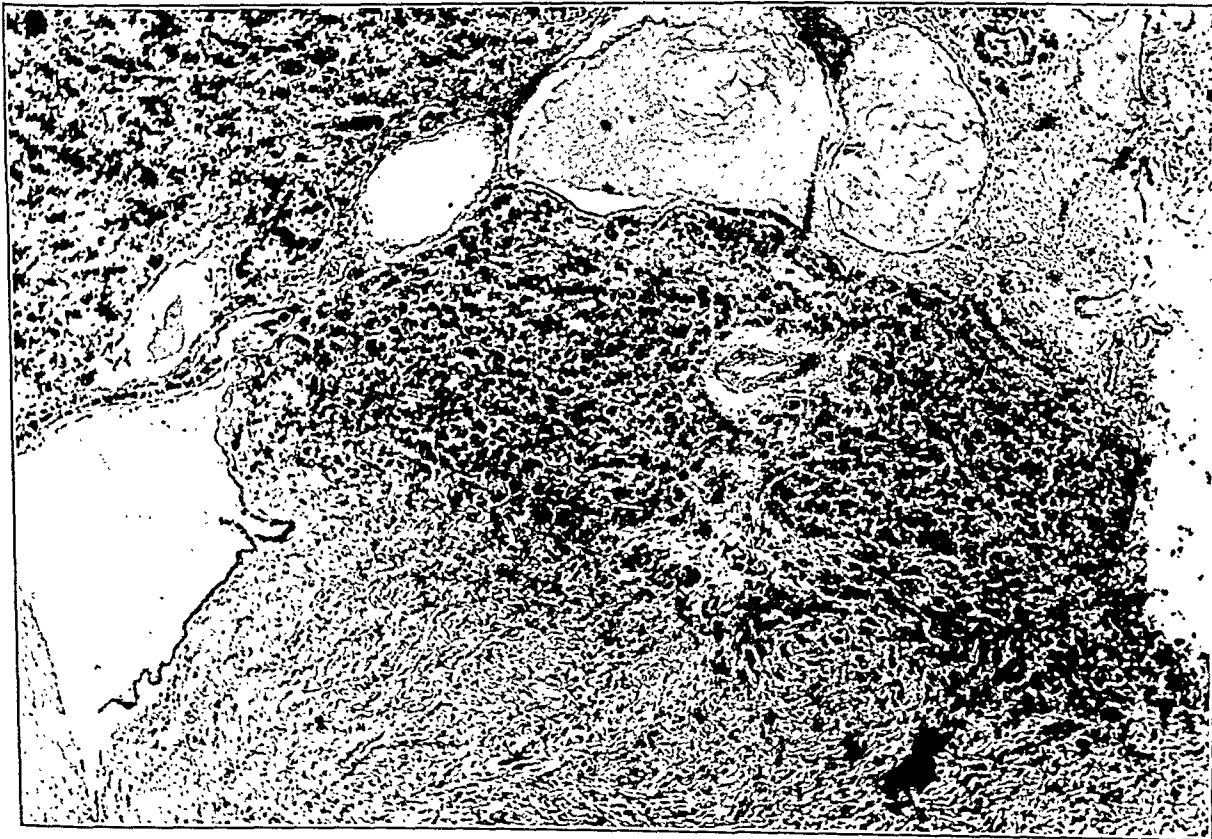
PLATE 65

FIG. 24. (Case 10.) Zone of activated basophils from pars intermedia from a case of essential hypertension (mag. $\times 60$) in a man of middle age.

FIG. 25. (Case 11.) Showing (mag. $\times 60$) posterior lobe invasion in a 60 year old man with hypertension and atherosclerosis.



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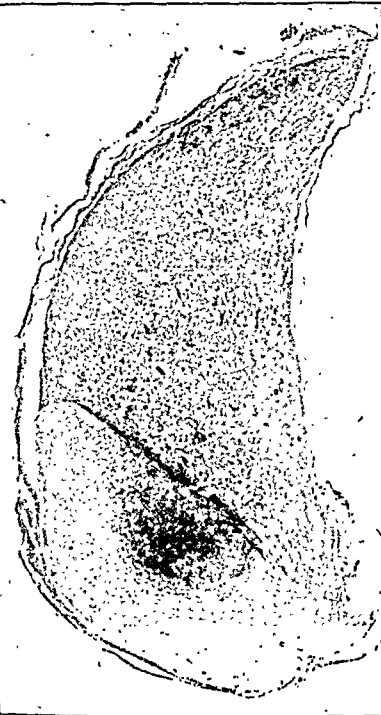
Cushing

Hyperactivation of the Neurohypophysis

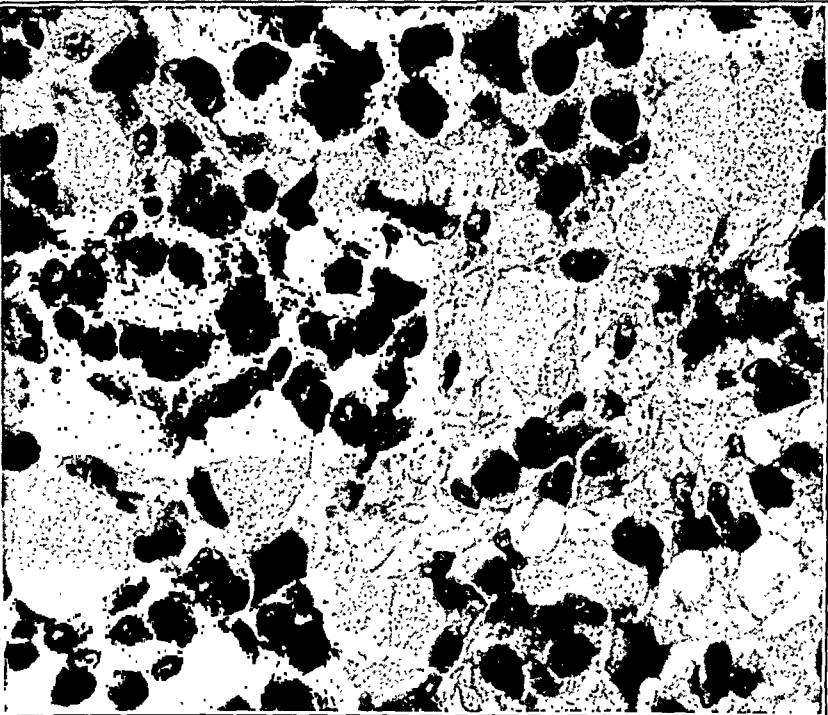
PLATE 66

FIGS. 26 and 27. (Case 12.) Sagittal section (mag. $\times 8$) of small gland from 67 year old woman with marked hypertension and heavy posterior lobe invasion. In Fig. 27 (mag. $\times 600$) are seen well preserved masses of holocrine secretion showing ghosts of swollen nuclei.

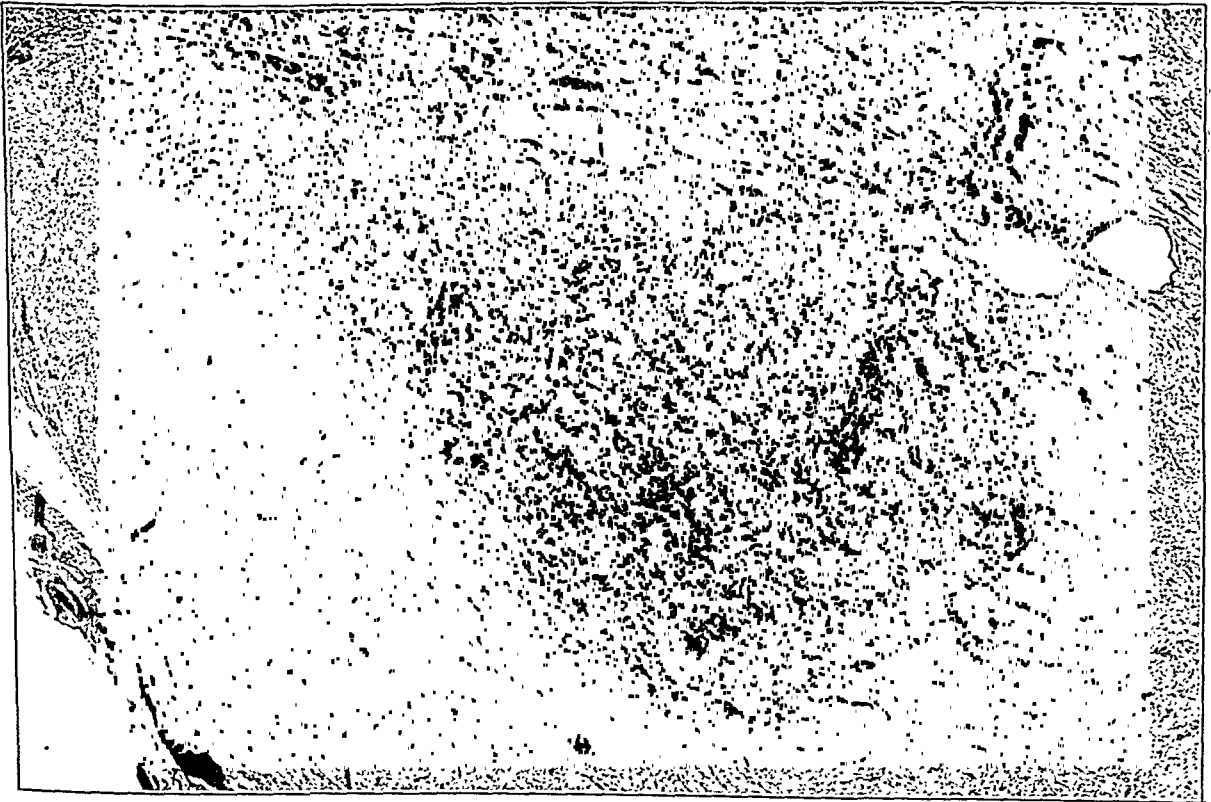
FIG. 28. (Case 12.) Showing (mag. $\times 40$) the area of massive invasion easily visible to the unaided eye (*cf.* Fig. 26).



26



27



28

Cushing

Hyperactivation of the Neurohypophysis

PLATE 67

FIG. 29. (Case 13.) Sagittal section (mag. $\times 8$) from gland of an aged woman with atherosclerosis, showing massive posterior lobe invasion. Arrows point to position of cleft.

FIG. 30. (Case 13.) Showing on higher magnification ($\times 30$) the full extent of the infiltration. A corner of pars distalis shows in the lower left corner.



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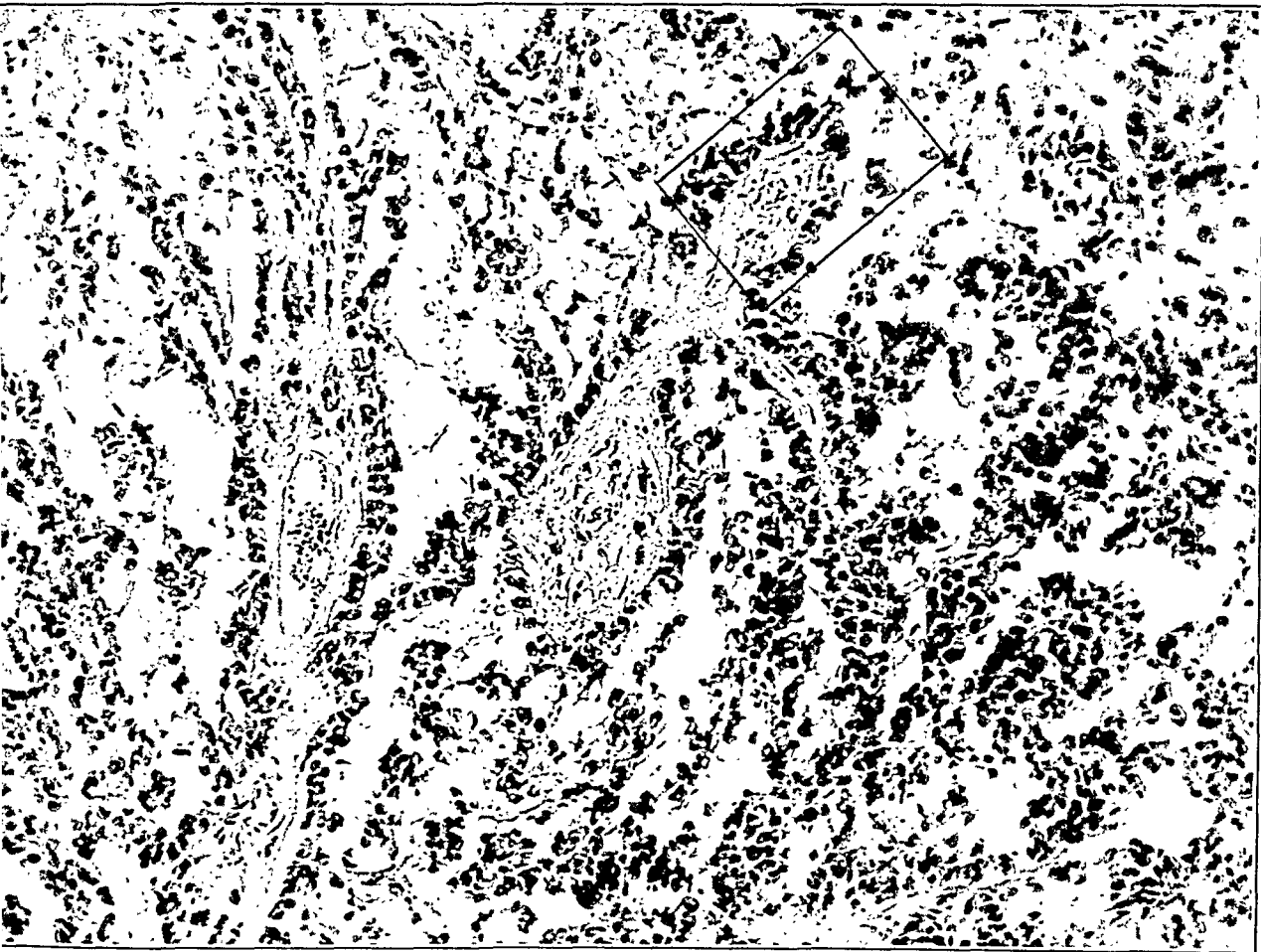
Cushing

Hyperactivation of the Neurohypophysis

PLATE 68

FIG. 31. (Case 13.) Posterior fringe of invading elements.

FIG. 32. (Case 13.) Squared area from Fig. 31 (mag. $\times 850$) to show typical vacuolated cytoplasm of basophilic elements.



31



32

Cushing

Hyperactivation of the Neurohypophysis

PLATE 69

FIGS. 33 and 34. Moderate invasion ($\times 60$) in two cases of accidental death. (Kindness of Professor Turnbull.)



33



34

Cushing

Hyperactivation of the Neurohypophysis

CARDIOVASCULAR RENAL CHANGES ASSOCIATED WITH BASOPHIL ADENOMA OF THE ANTERIOR LOBE OF THE PITUITARY (CUSHING'S SYNDROME)*

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(From the Pathological Departments of Tufts College Medical School, Boston, Mass., Guys Hospital, London, England, and the Peter Bent Brigham Hospital, Boston, Mass.)

This brief report is submitted through the kindness and encouragement of Dr. Cushing of the surgical department of the Peter Bent Brigham Hospital in Boston, and Drs. Bishop and Close of the pathological department of Guys Hospital in London, who have allowed us to make histological studies of the kidneys and other organs from patients showing clinical signs of pituitary basophilism.

When one groups together the cases reported of basophil adenoma of the pituitary in order to study and to unravel the complex clinical syndrome one finds recurring with a marked regularity certain signs and symptoms indicative of cardiovascular renal pathology. Emphasis has already been focused on this point by Cushing^{1, 2, 3} and others, but we can find no mention in the literature as to just what type of cardiovascular renal pathology occurs in such cases. Is it possible that it is merely a coincidence that a cardiovascular renal picture should be found in patients showing this rather rare disease, or are we dealing here with a cardiovascular renal problem that is definitely an intrinsic part of the syndrome of pituitary basophilism?

In a group of patients showing pituitary basophilism, recently reported by Cushing, none had passed middle life but the blood pressure, both systolic and diastolic, was elevated and at times associated with headache, blurring of vision and retinal hemorrhages. In many cases years passed before signs and symptoms suggesting renal pathology appeared, and then they were sometimes transitory and variable. A clinical study of the "*formes frustes*" of pituitary basophilism offers little help in identifying the nature of the cardiovascular renal lesion associated with basophil adenoma, where an elevated blood pressure together with a large heart may be the only noteworthy finding. A study of the advanced cases, on the other

* Received for publication December 8, 1933.

hand, offers a possible key to this solution. In such patients one may find in addition to the hypertrophy of the left ventricle and hypertension defective excretion of phenolsulphonephthalein, an elevation of the non-protein nitrogen of the blood, a fixed specific gravity of the urine, a failure to dilute and concentrate fluids or to concentrate urea when taken orally, and lastly, one may find on examination of the urine a variable amount of albumin, hyaline and cellular casts, polymorphonuclear leukocytes, desquamated epithelial cells and varying quantities of erythrocytes. Edema may be present. Such findings as these have led to the following diagnoses: chronic nephritis, vascular nephritis, and granular atrophy of the kidney.

If one disregards for a moment such symptoms of pituitary basophilism as adiposity, kyphosis, amenorrhea, hypertrichosis, a plethoric appearance of the skin, polycythemia and backache, and focuses on the cardiovascular renal problem alone, one sees at once a striking similarity to the clinical picture of malignant nephrosclerosis, as originally described in 1914 by Volhard and Fahr.^{4, 5} This is characterized by an elevated blood pressure, a large heart, an increase in the non-protein nitrogen in the blood, a diminution in the concentrating and diluting power of the kidney, polyuria, neuroretinitis and uremia. Edema may or may not be present. An examination of the urine in these cases will show albumin, granular, hyaline and cellular casts, polymorphonuclear leukocytes, and frequently frank blood. One may find similar signs and symptoms in chronic glomerulonephritis, but the course of both disease and the relation and sequence of symptoms to one another in chronic glomerulonephritis and malignant nephrosclerosis differ. In the latter disease, early and even in more advanced cases the cardiovascular symptoms stand far in the foreground. An elevated blood pressure and left ventricular hypertrophy that may by chronic glomerulonephritis be slight or absent are developed in patients with malignant nephrosclerosis to a remarkable degree.

In July of the summer of 1933, while visiting the pathological laboratory at Guys Hospital, London, two of us with Dr. Osman had the opportunity to study histologically tissue from a case of basophil adenoma of the anterior lobe of the pituitary, which had recently been reported by Bishop and Close.⁶ To our surprise the histological picture was similar to that of malignant nephrosclerosis. On returning to America, Dr. Cushing, who has long been interested

in this same problem, allowed us to study sections from the kidney from one of his cases of basophilic adenoma of the pituitary,* and this, like the case of Bishop and Close, showed without question the histological findings of malignant nephrosclerosis.

Malignant nephrosclerosis, neither clinically nor at the autopsy table, is a common disease: in contrast to the frequency with which one meets patients with benign essential hypertension, malignant nephrosclerosis is rare. The purpose of this paper, however, is neither to describe nor to discuss in detail the clinical and histological changes of malignant nephrosclerosis, but rather to point out an extremely interesting clinical and pathological finding, namely, the presence of malignant nephrosclerosis in two patients with basophil adenoma of the anterior lobe of the pituitary.

CASE REPORTS

CASE 1. C. P. (case reported by Bishop and Close⁶), was a normal child until the age of 11 years, when she stopped growing and began to gain weight. She developed a ruddy complexion and her hair began to fall out. Menstruation began normally at the age of 14, but after a year the periods ceased, and except for 3 consecutive months, when she was 18 years of age, she suffered from amenorrhea. From the age of 14 onward she experienced severe headaches which occurred regularly every month. Six months before death the sight of the left eye became affected and 2 months later symptoms of excessive thirst and polyuria became manifest. There was also frequent backache.

At the age of 22, in November 1930, she was admitted to Guys Hospital. She was kept under observation for some time and was then discharged, but was readmitted a short time before death. On admission the most striking features were the very red complexion, dry scaly skin, hairiness of the face, chest and abdomen, and the stunted growth. A beard sufficient to necessitate the use of a razor was present. There was a patchy red erythema localized particularly to the left arm. She was slightly knock-kneed and there was a deformity of the left wrist and right hand. A radiogram showed a fissured fracture of the lower end of the left radius and a rarefaction without evidence of inflammation of the head of the fourth right metacarpal bone. While in the hospital the patient slept badly and complained mainly of thirst. Occasionally there was a feeling of suffocation. The headaches were troublesome and on many occasions the blood pressure was as high as 300 mm. Hg. After she had been in the ward for about a fortnight she had the first of a series of fits, which were relieved on three occasions by venesection, while lumbar puncture was performed about twice a week. Her intelligence was in no way impaired although her illness worried her a great deal, and she frequently resorted to tears. Her eyesight troubled her and there was a marked degree of retinitis with silver wire arteries and a scotoma of the left eye. Examination showed the heart to be slightly enlarged to the left with a loud aortic second sound. The average

* See Ref. 3, page 521.

blood pressure readings were 250/180 mm. Hg. The hemoglobin was 95 per cent. Records of the blood picture are unfortunately not available but it is believed that a red cell count was never higher than 5,000,000. The blood urea was 43 mg. per 100 cc. Blood sugar tolerance test showed delayed return to normal with a high fasting figure (0.14 gm. per 100 cc.). The blood sugar went up to 0.25 per cent and was still raised after 2 hours (0.21 per cent). The blood cholesterol was also slightly above normal (0.185 per cent instead of 0.150 per cent). The Wassermann reaction was negative. The serum calcium figure was within normal limits.

An investigation of the urine showed albumin and sugar to be present but no acetone. Pus, fatty and hyaline casts were also demonstrated. The concentrating power of the kidney was slightly defective. Shortly after the second admission to the hospital signs of acute edema of the lungs developed suddenly and death occurred.

When we examine this report and focus our attention on the cardiovascular renal problem alone, we find a young person with marked hypertension and a large hypertrophied left ventricle, suffering from headaches and disturbances in vision. An examination of the eye grounds revealed a marked degree of retinitis. The blood urea was considerably elevated and the blood cholesterol was above normal. The urine showed albumin, casts of various sorts and defective concentration. Surely from such findings our clinical diagnosis would rest between chronic glomerulonephritis and malignant nephrosclerosis, and when one considers the sequence of events the latter diagnosis is much more likely.

Postmortem Examination

External examination of the cadaver showed an obese, stunted body with hair over the entire abdomen and on the chest. There were many hemorrhages beneath the skin of the limbs. The pituitary fossa and its contents were preserved. There was considerable edema of the lungs with an excess of frothy fluid in the bronchi. The left ventricle was hypertrophied and dilated, and the heart muscle pale and mottled. Extensive arteriosclerosis was found throughout the vessels. The liver was passively congested and the pancreas greatly reduced in size. The spleen and suprarenals were normal. The kidneys, which were rather small, showed scarring of the surface, which had a "flea-bitten" appearance. There were several hemorrhages beneath the mucosa of the bladder. The uterus was infantile in type while the ovaries were small and without visible evidence of Graafian follicles.

Again to review this report, focusing our attention once more on the cardiovascular renal problem, we have marked hypertensive hypertrophy of the left ventricle, diffuse arteriosclerosis, a granular kidney, with hemorrhages into the kidney, bladder and skin, and finally terminal pulmonary edema, which is so commonly seen in death associated with malignant nephrosclerosis.

Microscopic Examination

The entire kidney is severely injured by a chronic diffuse pathological process involving the vessels, the glomeruli, the tubules and stroma, leading to an almost complete reconstruction of the parenchyma and sclerosis of the interstitial tissue. The vessels show a varied picture. In a branch of the *renal artery* the intima is thickened by a narrow polster made up of a fibrillary ground substance staining blue by the Mallory anilin blue stain and showing flecks, shreds, and clumps of fibrin near the endothelial surface. The most recently formed portion of this polster is adjacent to the endothelium where it appears to have an almost semifluid appearance in which fibrils are poorly formed and appear more like lines and threads of coagulated protein, which show no definite order or arrangement. Farther away from the lumen this polster varies in structure and takes on rather a band-like arrangement in which cells are separated by well formed collagen fibrils. Just inside the original elastic interna there is seen in the Mallory anilin blue stain a bluish yellow, clear hyaline band, very narrow and showing fine reddish dots. This lamella in the elastic tissue-stained preparation is moderately positive so that we have here probably the beginning of a second elastic lamella arising in ground substance. The original elastica interna is fragmented, stains irregularly and in places is impregnated with calcium. The media of this vessel shows two interesting features: first, a hypertrophy of the individual muscle fibers, and second, a great increase in fibrillary ground substance between the muscle fibers. The adventitia is little changed. This vessel is large, the wall is generally thickened and the lumen is larger than normal. As one follows the large artery to the *interlobar* branches one finds in the latter a similar change, namely, a slight intimal and medial thickening, and the lumen is wider than that of a normal interlobar artery in a patient of the same age. In places the smooth muscle fibers show regressive changes, with disintegration and disappear-

ance. In the *arcuate arteries* one again sees this vascular hypertrophy, with dilatation of the lumen and regressive changes in an already hypertrophied muscular media. The *lobular arteries* show a striking change from the three sizes of vessels already described (the renal, interlobar and arcuate). Here the media is devoid of muscle fibers. It is represented merely by a blue-staining fibrous wall that in some places is not clearly defined from the surrounding stroma. Here and there an occasional muscle fiber is still recognizable. The basement membrane is not swollen and, except for areas where it has disappeared or ruptured, appears unchanged. Between the endothelium and the basement membrane one finds a bluish fibrillary substance (using the Mallory anilin blue stain) often containing delicate fibrin threads and not infrequently filled with large coarse clumps of fibrin and red blood cells. Occasionally fibrin and red blood cells may be traced throughout the wall. In these vessels the lumina are greatly narrowed and not infrequently obliterated by fibrin thrombi, or simply by the accumulation of subendothelial ground substance or the accumulation of nests of large "foam cells." There is no lamellar elastosis in these lobular arteries. The *afferent arterioles* to the glomeruli show changes resembling those in the lobular vessels just described, with necrosis of the wall and fibrin thrombi in the lumen on the one hand and old healed sclerotic vessels with occluded lumina on the other. The type of sclerosis here is characterized by a lamellar arrangement of fibrous tissue beneath the endothelium in which cells and fibrils form concentric whirls within one another, greatly narrowing the lumen.

The *glomeruli* show a varied picture. About 70 per cent of those seen in the sections examined show a rather characteristic ischemia, together with an increase in cells and ground substance, and in contrast to the normal glomerulus they are large. There are clusters of glomeruli usually just beneath the capsule which show the simple hyaline transformation with thickening of the capsule. The most interesting glomerular lesion is the fresh fulminating degenerative and inflammatory lesion associated with aneurysmal dilatation of the capillaries, hemorrhage and fibrin within the lumina and throughout the ground substance, so characteristic of malignant nephrosclerosis. Where this lesion is somewhat older there is proliferation of both endothelium and epithelial cells with desquamation and adhesions between the capillary loops and from the

capillary loops to the capsular wall. This may be associated with proliferation of the cells along the capsular wall. Where the lesion has healed many of the cells have disappeared and the glomerulus itself may no longer be easily recognizable. One finds acute lesions, others that are healing, others that have healed, and still others showing recurrent fresh lesions in glomeruli that have long ago healed. These lesions appear at times isolated and at times in small clusters of glomeruli fed by the same lobular artery.

The *tubules* show as variable a histological picture as that in the arteries and glomeruli, and like the changes in both of those one finds both fresh and old lesions. The most interesting change is the almost complete absence of well differentiated proximal convoluted tubules. A search for such clearly recognizable proximal convoluted tubules in which one seeks a rather characteristic type of epithelium reveals only here and there small scattered islands; and the cells lining these show albuminous granular degeneration, hyaline droplet degeneration and, in places, necrosis. The great majority of tubules are small, collapsed and poorly differentiated, and lined by small atrophic cuboidal cells. The lumina are narrow and contain little precipitated protein. A third type of tubule commonly found also lacks differentiation and is characterized by marked dilatation with endothelial-like cells lining the wall. Mitoses in such tubules are quite common and their lumina contain precipitated albumin, a few polymorphonuclear leukocytes and red blood cells. In a few areas the tubules have entirely disappeared. This, however, is a rare finding and is best seen at the tips of the papillae.

The *stroma* in both cortex and medulla is increased. When stained by the Mallory anilin blue stain it appears as a blue-staining, finely fibrillar ground substance, which is most marked in areas where the tubules are small and atrophic or where they have disappeared entirely, but is also present, though to a much less degree, about the tubules that form the islands of still recognizable proximal convoluted tubules. This material has the same structure, stain and character as the material beneath the endothelium of the arteries. There are small foci of the lymphocytes limited largely to the areas of sclerosis.

The *veins and capillaries* are dilated, especially the capillaries surrounding the tubules, but there are no hemorrhages from these vessels into the stroma.

The *basement membrane* in vessels, glomeruli and tubules shows a series of interesting changes. In the arterioles, as already mentioned, it is in places broken up and absent. In the glomerular capillaries it is separated from the endothelium by a newly formed, finely fibrillar ground substance. The basement membrane forming the glomerular capsules is here and there thickened. On the tubules, especially where they have collapsed, the basement membrane is thickened and somewhat irregular, and here and there one finds a fine fibrillary ground substance between the collapsed epithelium and the original basement membrane—a picture corresponding very closely to the accumulation of fibrillary ground substance beneath the endothelium in the smaller vessels.

To summarize these histological changes, we find a severely damaged kidney; the larger arteries show vascular hypertrophy, the smaller show regressive changes, necrosis, thrombosis and hemorrhage. Some show healed lesions, others fresh lesions and still others show chronic lesions, occasionally with fresh hemorrhage superimposed. The glomeruli for the most part are still readily recognizable, being large, anemic and rich in cells. There are areas in which the glomeruli show acute, healing, healed, chronic and recurrent degenerative and inflammatory processes characterized by hemorrhage, necrosis and cellular proliferation leading in places to half-moon formation within the capsule. The tubules are severely injured; only nests of recognizable proximal convoluted tubules are present. The majority are small and atrophic while others are dilated, poorly differentiated and filled with coagulated protein, desquamated epithelial cells, polymorphonuclear leukocytes and erythrocytes. The stroma is diffusely increased, the veins and capillaries are congested. Such findings as these are neither compatible with chronic glomerulonephritis nor with benign nephrosclerosis, but are characteristic of chronic malignant nephrosclerosis which has been progressing with remissions for several years.

In keeping with this picture of malignant nephrosclerosis the spleen, liver, intestine and ovary reveal the same variation and character of histological changes in the smaller blood vessels. In the spleen many of the small arterioles show fibrin throughout the wall and occlusion of the lumen, and still others show marked swelling of the basement membrane with partial occlusion of the lumen. In the ovary these changes are especially marked where

some of the small vessels are almost completely transformed into walls of fibrin.

CASE 2. H. P. (case reported by Cushing³), an unmarried, white female, 33 years of age, entered the hospital with a history of two periods of amenorrhea. The first attack was of 1 year and 8 months duration, occurring when the patient was 20 years of age. The present attack began 1 year and 3 months ago. She was born of healthy parents and attained normal adolescence at the age of 13, when she later grew into an intelligent, vigorous and ambitious young woman. She entered college at 18 but became unhappy there and withdrew at the end of the second year. She ascribes this to restlessness and emotional instability. In 1919, when she first ceased to menstruate, she developed a ravenous appetite, gained weight rapidly, particularly in the face and abdomen. During the summer of 1919 she broke her ankle. Purplish striae of the body and arms began to appear at that time. In December of 1919, she found herself easily fatigued and acquired a definite polyuria and polydipsia. At the same time headaches occurred, with blurred vision, tinnitus, dizziness and numbness of the hands.

Toward the end of February 1920, because of a sudden fainting attack, she came under the care of Dr. E. P. Joslin, who found there was a moderate hyperglycemia with glycosuria, and a basal metabolic rate of -30 per cent. On March 13, 1920, she was first seen briefly in consultation with Dr. Joslin. The facial hypertrichosis and the peculiar disposition of the adiposity with extraordinarily widespread striae atrophicae, associated with a moderate hypertension of 140/100, indicated a polyglandular syndrome. By January 1921, she had become increasingly hirsute and "bloated" in appearance. At this time she entered the Neurological Institute in New York where she was given baths, exercises and glandular preparations. After 4 weeks the weight was reduced, the hirsuties had disappeared and normal menstruation was resumed. From this time, for a period of 5 years, she continued under various combinations of glandular treatment and regarded herself as reasonably well.

In 1926 the face again began to get heavily bearded, necessitating the use of a razor. A year later tonsils, adenoids and impacted wisdom teeth were removed and it was noticed that the blood pressure was high, 155/115. In 1929 she had a "nervous breakdown," and the following year, while being studied at the Evans Memorial Hospital, it was noticed that the urine showed some albumin and an occasional hyaline cast with a normal phthalein test. She had a low sugar tolerance, a fluctuating hypertension, a basal metabolic rate of -14 per cent and cardiac enlargement. In January 1931 the menstrual periods, after having been essentially regular for 10 years, ceased, and in July she was found to have a marked hypertension varying from 220 to 250 systolic.

In 1932 she fell and fractured the humerus. The following summer polydipsia, occipital headaches, palpitation, shortness of breath and swelling of the feet and ankles were present. The fatness of the face and shoulders, dryness and pigmentation of the skin, cyanosis of the dependent hands and feet had markedly increased. It was observed that large ecchymoses would follow the slightest bruise and that a cut or scratch would bleed excessively. At this juncture, in October 1932, she was referred to the Peter Bent Brigham Hospital for study.

The patient was a rather tall woman, 5 feet, 9½ inches, weighing 63.5 Kg., with a peculiar moon-shaped, recently shaven face with clipped eyebrows. The eyes were puffy and there were posterior cervical and supraclavicular fat pads. She was not appreciably round shouldered and though not particularly abdominous the parietes were somewhat pendulous and flabby. The extremities did not participate in this adiposity. Over the arms, axilla, breasts, abdomen, hips, groins and thighs were an extraordinary number of broad, pale striae atrophicae. The lower extremities showed marked pigmentation and scarring of the dry and scaly skin, with several large and fading ecchymoses from recent trivial contusions.

The blood pressure averaged 220/170, the urine showed a trace of sugar and of albumin with no renal elements. There was a variable polyuria amounting to about 3 liters. The basal metabolic rate was -10 per cent. The detailed blood examination showed 4,720,000 erythrocytes, with a hemoglobin (Sahli) of 106 per cent. The non-protein nitrogen was 46.97 mg. and the cholesterol 192.3 mg. per cent. Roentgenograms showed slight diffuse atrophy of the vertebral bodies without collapse or deformity, normal detail of the cranial bones, a sella tursica of normal dimensions but hazy outline, and multiple small tiny shadows in both flanks suggesting renal calculi — a common finding in hyperparathyroidism. The patient was transferred to the Huntington Hospital where, through the kindness of Dr. Aub, her elimination was thoroughly studied. He reported essentially normal blood content for calcium phosphorus and phosphatase and normal elimination of both calcium and phosphorus. She showed a low sugar tolerance and a high nitrogen output, as shown by an average loss of 6.7 gm. daily on a balanced diet containing 56 gm. of protein.

On readmission the pituitary body was irradiated on 4 successive days without any immediate effects. She was discharged Nov. 12, 1932, and returned home and resumed her usual activities. On December 3 she retired about midnight, waking about an hour later with dyspnea and increasing cyanosis. She died 12 hours later from what was supposed to be acute pulmonary edema.

When we study this patient in retrospect from a cardiovascular renal standpoint we find that in 1919, when the patient was 20 years old, she began having headaches, blurred vision, tinnitus, dizziness and numbness of the hands. Several months later we find the blood pressure slightly elevated, 140/100. Six years later the blood pressure was again examined and had reached 155/115, and the urine contained albumin and casts. The heart at this time was enlarged. Five years later we find the hypertension had increased to nearly 250 systolic and a year later there were occipital headaches, palpitation, shortness of breath and swelling of the feet and ankles, and the patient observed that large ecchymoses would follow the slightest bruise. The physical examination at the time of hospital entry in 1932 substantiated this high blood pressure with some albumin in the urine. There was a variable polyuria and the non-protein nitrogen and cholesterol values were increased and the

nitrogen output showed an average loss of 6.7 gm. daily on a balanced diet of 56 gm. of protein. Finally, death came suddenly, probably to be explained on the basis of pulmonary edema.

From such a summary our attention is primarily focused upon the cardiovascular problem, with its hypertension, enlarged heart, headaches and disturbances in vision. The disturbances in renal function with albumin, elevated non-protein nitrogen and cholesterol stand rather in the background and yet the sequence of events must be carefully considered, and a clinical diagnosis of benign nephrosclerosis with beginning renal decompensation or malignant nephrosclerosis must be considered. The tendency to bleed when slightly bruised is found more commonly associated with the latter diagnosis.

Postmortem Examination

An autopsy was performed by Drs. Schulz, Hass and Cushing, and only the cardiovascular renal changes will be mentioned here. The heart was enlarged (695 gm.) and the large arteries, including the aorta, showed an advanced degree of atherosclerosis. The kidneys had slightly adherent capsules and on section minute calculi were visible in the calices.

Microscopic Examination

The kidney is finely granular, the capsule thickened, and small hemorrhages are seen over the surface. The kidney as a whole shows only moderately severe changes, the most important of which appear in the cortex and are somewhat irregularly distributed. There is a destruction of kidney tissue and a new formation of tubules with a general reconstruction of the normal architecture. The arteries show a variety of interesting changes. The large *interlobar* arteries show marked medial hypertrophy with large, well preserved muscle fibers. The intercellular ground substance is not remarkably increased. The internal elastic lamina is intact and stains well. The intima is only slightly thickened, showing a lamellar connective tissue thickening. Practically the same changes are found in the *arcuate* and larger lobular arteries; that is, we have in these vessels a form of vascular hypertrophy with an enlargement of the vessel, a thickening of the vessel wall and a lumen larger than that of a normal person of the corresponding age. In the smaller *lobular*

arteries one sees a gradual disappearance of the muscle fibers from the largest to the smallest arterioles. The vessels are abnormally large and the media shows replacement by connective tissue. The internal elastic lamina is still preserved, and the basement membrane of the arterioles is here and there remarkably swollen, irregular, and when stained with the Mallory anilin blue stain appears reddish yellow. In other arterioles the basement membrane is unchanged and between the endothelium and this basement membrane there is an accumulation of watery-like material forming in places a very fine network which, when stained with the Mallory anilin blue stain, appears distinctly blue. This accumulation beneath the endothelium leads in places to almost complete obliteration of the lumen. In other vessels of similar caliber, where the process is older, one sees a lamellated arrangement of cells and connective tissue in which spindle-shaped cells appear drawn out and separated by narrow bundles of collagen, giving a characteristic "onion-like" picture. A still more striking change, though somewhat rarely seen, is the presence of fibrin and red blood cells within the wall, which at times is completely necrotic. Nests of fatty endothelial cells beneath the endothelium and fibrin in the lumen may complicate the picture. Some of the arterioles to the glomeruli are scarcely recognizable. The lumina of the vessels comprising the smaller branches of the vascular tree are greatly reduced. It is not infrequent to find extensive vascular changes without a corresponding change occurring in the capillary loops of the glomeruli.

The *glomeruli* are relatively little changed. By count, between 90 and 95 per cent are still preserved, the most of which, however, are large, ischemic and show an increase in cells and intercellular substance. Here and there, usually occurring in small groups of two and three, glomeruli show simple hyaline transformation with connective tissue thickening of the collapsed capsule. Here and there, even in areas showing rather advanced atrophy of the tubules, one finds well preserved glomeruli, rich in blood, showing neither cellular nor intercellular changes. The most striking change is that so characteristic of malignant nephrosclerosis, namely, the capillary dilatation, hemorrhage into the capsular space and fibrin occluding the capillary lumina and extending out into the somewhat loose intercellular basement membrane and ground substance. Regressive changes in the epithelial cells are occasionally found associated with

proliferation and desquamation. Glomeruli showing these changes are infrequent. They appear singly and in small groups. Somewhat older changes, including the chronic and the healed lesions with simplification of the dilated capillaries, adhesions of the capillary loops with each other, and with the capsular wall, and even half-moon formations are also found, but only rarely.

The *tubules* show the usual varied picture, such as is seen in malignant nephrosclerosis. Unlike the former case, however, many of the proximal convoluted tubules are still moderately well preserved, showing the characteristic type of epithelium. They are moderately hypertrophied and in places show regressive changes leading at times even to necrosis. Most of the tubules are changed. They are small, atrophic, collapsed and bordered with small cuboidal, undifferentiated epithelial cells often containing in the lumina small hyaline casts. Here and there and usually occurring in islands are nests of tubules showing dilatation; such tubules are bordered with elongated endothelial-like cells showing frequent mitoses, and frequently contain remnants of necrotic desquamated cells, precipitated albumin and polymorphonuclear leukocytes. Stains for bacteria are negative. The necrotic cells are incrustated with salts often rich in iron and in places surrounded by large multinucleated foreign body giant cells. It is interesting that even in this group of reformed tubules hyaline droplet degeneration is already present in the cells. In the medulla where casts incrustated with iron have remained fixed the surrounding epithelium has totally disappeared, leaving this foreign body surrounded by connective tissue.

The *veins* throughout the kidney and the *intertubular capillaries* are unusually dilated and one sees very well the very close relation that the intertubular capillaries bear to the tubules, being separated only by a basement membrane, an anatomical relation similar to that of the capillary tufts of the glomerulus. Occasionally one sees petechial hemorrhages into the stroma of both cortex and medulla from these dilated capillaries.

The *basement membrane* of the arterioles is markedly swollen and rich in lipoids but this kidney, like the former, shows no double refractile fat. Along the capillary loops of the glomerular tufts the basement membrane seems loose, lax, and increased and fibrillated, but not swollen like that of the arterioles. The basement

membrane forming the capsule in places is rather coarse but quite uniform and shows no papillary bulging. Along the tubules, and especially those that are collapsed, it is swollen and irregular, and here and there fluid-like material slightly fibrillar has collected between the collapsed epithelium and the basement membrane.

The *interstitial tissue* is unevenly increased and shows foci of lymphocytes, especially in areas of tubular atrophy and disappearance. In the medulla the stroma is diffusely increased, yet there is practically no total disappearance of tubules.

To summarize these histological changes, we find a moderately damaged kidney with changes involving the blood vessels, glomeruli, tubules and stroma. The lesions for the most part are chronic and fairly advanced. From the character and extent of the histological lesion alone there is no basis for one to believe that either through the vascular, glomerular, or tubular changes this patient should suffer from severe renal insufficiency. The lesions, being largely of a chronic nature, warrant the diagnosis of a slowly progressive type of malignant nephrosclerosis. This is substantiated by the finding of similar, if not more severe, lesions of the same character in many of the other organs of the body.

DISCUSSION

A study of the etiology of malignant nephrosclerosis has, since the work of Volhard and Fahr, occupied the attention of many investigators. A recent paper by Schürmann and MacMahon⁷ reviews the work that has already been done up to the present time. Without question it would appear that the anterior lobe of the pituitary, and especially the secretion of the basophilic cells, may, in some cases, play a very important rôle in the etiology of this important cardiovascular renal syndrome. Most cases of malignant nephrosclerosis, however, are not characterized by such signs of pituitary basophilism as adiposity, disturbances in secondary sexual characteristics and osteoporosis.

For years clinicians and pathologists considered the bony changes of osteitis fibrosa deformans and osteodystrophia fibrosa cystica as being one and the same fundamental disease. Only with the discovery of the important rôle played by the parathyroid in cystic disease of bone were the clinical and histological differences in these two diseases accepted as definite and distinct entities. Now the

question may be asked — can we distinguish a particular group of cases of malignant nephrosclerosis clinically or histologically which specifically belong to the syndrome of basophil tumors of the anterior lobe of the pituitary? From a study of fifty cases of malignant nephrosclerosis one finds variations in the clinical and pathological picture, but clinically these cases of malignant nephrosclerosis associated with typical signs of pituitary basophilism, as described by Cushing, belong to a very definite and distinct group. Comparing the vascular changes in these two cases of basophilic adenoma of the anterior lobe of the pituitary with the vascular changes in many other cases of malignant nephrosclerosis which did not show pituitary basophilism, we could find no single change or group of changes that would permit us to distinguish one case specifically from another.

SUMMARY

Two cases of basophilic adenoma of the anterior lobe of the pituitary, one reported by Bishop and Close and the other by Cushing, have been discussed again from a cardiovascular renal standpoint in which it is shown that the cardiovascular renal lesion present in these two cases corresponds to the picture originally described as malignant nephrosclerosis by Fahr.

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STUDIES ON INFLAMMATION

X. THE CYTOLOGICAL PICTURE OF AN INFLAMMATORY EXUDATE IN RELATION TO ITS HYDROGEN ION CONCENTRATION *

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For many years it has been known that the cytological sequence in acute inflammation is characterized in the earliest stages by an active emigration of polymorphonuclear leucocytes. After a time this is followed by an infiltration of mononuclear phagocytes. The latter have been designated by various names, the most satisfactory of which is perhaps that of "macrophage," originally suggested by Metchnikoff. In acute inflammation the polymorphonuclear cells that leave the circulating blood stream form the chief cellular constituents of the early exudate. The mononuclear phagocytes or macrophages increase in number in the later stages. These cells act as scavengers when the inflammatory irritant has been overcome. They engage actively in engulfing and digesting polymorphonuclear leucocytes, red cells, and various necrotic materials resulting from the acute inflammation. The orderly cytological sequence in the development of an inflammatory reaction was first pointed out by Borrel¹ and then by Durham² about forty years ago. The subsequent studies of Beattie³ extended considerably the original observations of Durham. This sequence is true of the majority of inflammatory reactions caused either by bacteria or by chemical irritants. It is noteworthy that during the first twenty-four hours after their inoculation into normal tissues both tubercle and typhoid bacilli produce the same type of cellular changes as do various forms of pyogenic bacteria such as *Staphylococcus aureus*.^{1, 4, 5} The difference in the leucocytic response found with various types of inflammatory irritants seems therefore to be one of degree rather than of kind.

No adequate explanation has been offered for this fundamental process. A number of years ago various investigators, particularly

* This study was aided by a grant from the DeLamar Mobile Research Fund.
Received for publication September 28, 1933.

Opie, studied the action of intracellular proteolytic enzymes from leucocytes of an inflammatory exudate.^{5,6} Müller,⁷ and subsequently Opie, showed that polymorphonuclear leucocytes contain an intracellular enzyme that acts in a slightly alkaline or neutral medium, but is almost wholly inactive in an acid reaction (0.2 per cent acetic acid). Opie designated this intracellular enzyme "leucoprotease." The action of this polymorphonuclear enzyme occurs only within the leucocyte, for in the plasma of an inflammatory exudate its activity is inhibited owing to the action of anti-enzymes. The earlier observations of Opie on the presence of antiferments inhibiting the action of leucoprotease have been recently confirmed by Weiss.⁸ Opie furthermore demonstrated that the mononuclear phagocytes that accumulate in the later stages of the inflammatory reaction contain an enzyme causing active digestion of protein in a weakly acid medium, but almost entirely inactive at a neutral or alkaline reaction. The enzyme of the mononuclear phagocyte has been called "lymphoprotease."

It is conceivable that particles in an inflammatory exudate prior to being phagocytosed by a given type of leucocyte may tend to have on their surfaces a hydrogen ion concentration approximating that of the intracellular proteolytic enzyme capable of digesting them. If this assumption is correct then it is to be expected that the inflammatory exudate in which such particles are immersed would gradually increase in its acidity concomitantly with the shift from polymorphonuclear to mononuclear phagocytes. The question arises therefore as to whether or not there is a correlation between the pH of the medium and the cytological picture during the development of an acute inflammatory reaction. In this connection it is to be noted that Opie recorded several measurements on the reaction of a pleural exudate.⁹ At no time during a 5 day period of the inflammatory process did the alkalinity of the exuded serum disappear; it was, however, less than that of the blood serum. It may be mentioned also that as the inflammation progressed there seemed to be a slight decrease in alkalinity. Since, however, no precautions apparently were taken to avoid loss of carbon dioxide during withdrawal and testing of the exudate, the validity of these measurements as absolute figures may be open to some question. Lord¹⁰ in his studies on proteolytic enzymes in the pneumonic lung concluded that during the course of the disease a gradual increase

in the hydrogen ion concentration of the exudate probably occurs. He conceived resolution to be the result of this increased hydrogen ion concentration, which eventually activated a proteolytic enzyme having a range of optimum reactivity at a pH of 6.3 and 5.2.

Rous found that death of small cell aggregates resulted in the development of an alkalinity of these cells, owing to seepage into them of alkaline body fluids.¹¹ He recognized that the chemical changes that take place in small necroses differ in important respects from those occurring in large masses of dead tissue. He was led by his observations to conclude that very pronounced inflammatory edemas yield alkaline fluids but that "inflammation, as such, conduces to local acidosis."

None of the studies mentioned has correlated the pH of the inflammatory exudate with its differential leucocyte count. The object of the present communication is to report data on the trend of the hydrogen ion concentration and to relate this to the cellular changes in an exudate obtained at various intervals from an acute inflammatory area. The relation obtained suggests that the prevailing hydrogen ion concentration may be an important factor in determining at a given time the cytological picture of an inflammatory exudate.

EXPERIMENTAL

Method: Pleural exudation was induced by the injection under ether anesthesia of 1.5 to 2 cc. of turpentine into the right chest of dogs.⁹ Several hours to 1 day following the injection of the irritant a sample of the exudate was withdrawn by means of a Luer syringe with a hypodermic needle. The latter was of large caliber and filed off at the end, in order to diminish the chance of injury to the lungs. To prevent coagulation several glass beads were placed in the barrel of the syringe. When in one experiment about 0.5 cc. of 0.1 per cent heparin in Tyrode solution was employed as an anticoagulant, essentially the same readings were obtained as with the use of glass beads. Upon withdrawing the sample of exudate the syringe was shaken quickly for a few seconds and several smears were made on coverslips and slides. The remaining part of the exudate was immediately transferred under paraffin oil into a test tube.

Measurements of the pH were always performed within a short interval after withdrawing the sample of pleural exudate. The bicolor system of standards, as described by Hastings and Sendroy,¹² was employed in determining the hydrogen ion concentration. These investigators had found close agreement when results obtained by this method were checked up with parallel electrometric pH measurements. They also had determined that the "salt and protein errors" were negligible. The standards prepared with phenol red as indicator covered a range of pH 6.7 to pH 8. In a few instances the pH of the exudate was found slightly below 6.7. The reading in such cases was obtained

TABLE I

The Hydrogen Ion Concentration and the Cytological Picture in Acute Inflammation

Dog No.	Interval between injection of irritant and removal of exudate	Differential leucocyte count of inflammatory exudate			pH of inflammatory exudate	Differential leucocyte count and pH of blood			
		Poly-morpho-nuclears	Lympho-cytes	Mono-nuclear phago-cytes		pH	Poly-morpho-nuclears	Lympho-cytes	Mono-nuclears
	<i>hrs. : mins.</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>			<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
4	19:15	78.0	2.0	20.0	7.45				
	43:45	78.0	1.0	21.0	7.23				
	67:15	87.0	1.0	12.0	7.23				
	93:08	31.0	3.0	66.0	6.97				
	115:00	26.0	2.0	72.0	6.95	7.07	83.0	7.0	10.0
5	22:57	79.0	3.0	18.0	7.15				
	47:00	9.5	0.5	90.0	6.8				
	71:15	2.0	0.0	98.0	6.6	7.4	77.0	13.0	10.0
7	24:10	68.5	7.0	24.5	7.4				
	48:30	72.6	3.4	24.0	7.35				
	72:45	75.7	6.0	18.3	7.45				
8	24:50	90.0	0.5	9.5	7.23				
	47:35	87.0	2.7	10.3	7.13				
	71:45	63.25	0.5	36.25	6.78				
	100:05	59.0	1.0	40.0	6.98	7.1			
3	23:00	90.0	1.5	8.5	7.05				
	48:10	53.5	1.0	45.5	6.75				
	72:40	7.0	1.0	92.0	6.6				
	95:40	10.0	3.0	87.0	6.65	7.23	71.0	7.5	21.5
2	23:42	69.3	8.3	22.3	7.4				
	47:37	74.3	2.6	23.0	7.4				
	71:12	83.0	2.0	16.6	7.25				
	95:22	88.3	0.6	11.0	7.25				
2-A*	19:35	7.23				
	43:35	13.0	3.0	84.0	6.6				
	67:10	3.5	0.5	96.0	6.7	7.23	77.0	10.0	13.0
10	6:15†	7.55				
	24:25	40.5	12.5	47.0	6.93				
	48:15	63.5	11.5	25.0	7.0				
	72:38	74.0	6.0	20.0	7.08				
	96:15	74.0	2.0	24.0	7.28				
	120:15	76.0	0.0	24.0	7.28				
	145:35	74.0	3.0	23.0	7.50				
	169:35	78.5	7.5	14.0	7.55				
11	24:15	26.0	9.75	64.25	6.98	7.28	69.0	18.0	13.0
9	52:55	72.0	6.0	22.0	7.05	7.05			
16	18:00	71.0	10.0	19.0	7.55	7.28	83.0	4.0	13.0
12‡	23:30	81.0	1.5	17.5	7.25				
	48:35	52.0	1.0	47.0	6.95				
14‡	23:25	90.0	0.66	9.33	7.2				
	47:20	64.0	4.5	31.5	6.95				
	75:20§	87.0	0.0	13.0	7.28				
13‡	24:06	87.5	2.0	10.5	7.4				
	40:02	77.5	2.5	20.0	7.5				
	71:45	74.0	6.0	20.0	6.68				
	95:30	10.0	8.5	81.5	6.8	7.45	76.0	6.0	15.0
15‡	23:25	86.0	1.0	13.0	7.35				
	47:15	42.5	2.0	55.5	6.8				
	75:40	17.5	3.5	79.0	6.75				

* Dog 2-A in Dog 2 is infected with suspension in the right pleural cavity 35 days after the first infection with the irritant.

† The exudate was of the exudate 6 hours and 25 minutes after the injection of the irritant; practically no leucocytes were found.

‡ A total of 4 cc. of a phosphate buffer mixture at pH 6.93 was injected in divided doses subsequent to the irritant.

§ The exudate was removed immediately after 75 minutes the animal.

¶ A total of 25 cc. of a phosphate buffer mixture at pH 6.93 was injected in divided doses subsequent to the first infection with the irritant.

by the use of bromcresol purple as indicator and represents only a first approximation. The thorough studies of Drury and Rous had shown that in the animal body, at least, the observed colors in tissues vitally stained with phenol red or bromcresol purple cannot be ascribed to indicator errors resulting from association of the phthalein with tissue materials.¹³ For this reason it is believed that the readings obtained by adding under oil 0.2 cc. of the pleural exudate to 4 cc. of a standard phenol red indicator solution (made up in saline and adjusted to about pH 7.4) are reasonably reliable and do not represent indicator errors. In several instances, in spite of an appropriate saline control tube, the turbidity of the exudate rendered readings somewhat difficult, so that centrifugalization for a few minutes had to be resorted to. The determinations were always made after the tubes had been immersed in a water bath at about 38° C for several minutes.

The differential leucocyte counts were made from smears on coverslips and slides. The cells were stained by the Wright method. As a rule several hundred cells were counted in each sample. In computing the percentage of polymorphonuclears and mononuclears cells were frequently encountered that were so degenerated as to render their identification difficult. These were not included in the final counts.

Samples of the pleural exudate were withdrawn daily and studied as described for a period not exceeding 1 week after the injection of the irritant. At the completion of an experiment the animal was anesthetized under ether and frequently a sample of blood was withdrawn from the femoral vein in a syringe containing about 0.5 to 1 cc. of 0.1 per cent heparin in Tyrode solution. The pH of the blood and its differential leucocyte count were determined. The administration of ether was continued until the death of the animal. A post-mortem examination was performed and specimens of the inflamed pleura and right lung were placed in 10 per cent formaldehyde for subsequent histological examination.

RESULTS

The results of all the 15 experiments performed are summarized in Table I. A cursory examination of the data shows that in 8 out of 12 animals in which the pleural exudate was studied from day to day, as the inflammatory reaction progressed, the hydrogen ion concentration changed from an alkaline to an acid pH. The change in the reaction toward a definite acidity occurs usually 2 or 3 days after the injection of the irritant. Concomitantly with this decrease in the alkalinity of the exudate there is a change in the differential leucocyte formula. The percentage of polymorphonuclears falls, whereas the percentage of mononuclear phagocytes correspondingly rises. The percentage of lymphocytes evidently plays no significant rôle in these cellular changes. Composite graphs of all experiments showing the parallelism in the fall of the pH and the drop in the percentage of polymorphonuclear leucocytes appear in Chart 1.

Since the percentage of polymorphonuclear leucocytes represents virtually the reciprocal of that of the mononuclears, the latter were not plotted on the chart. An examination of the data reveals that the percentage of polymorphonuclears predominates over that of the mononuclears whenever the pH is alkaline. A rise in the hy-

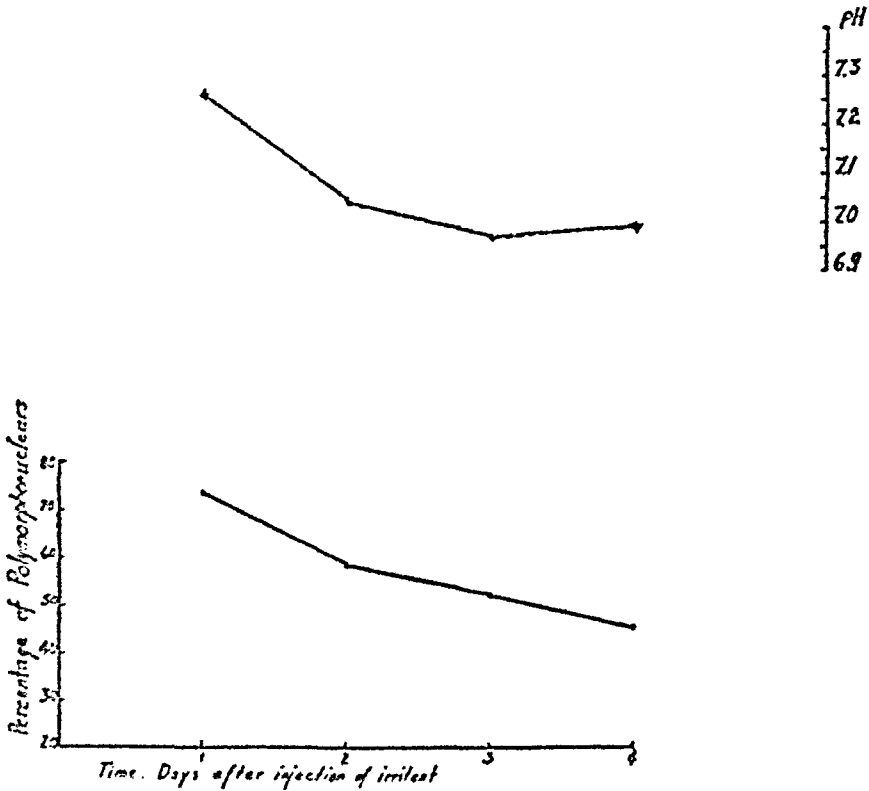


CHART 1

The hydrogen ion concentration in relation to the percentage of polymorphonuclear leucocytes in pleural inflammatory exudates. Composite graphs of 12 experiments.

-----+----- pH
 ————•———— Percentage of polymorphonuclear leucocytes.

drogen ion concentration is immediately or at least very soon followed by a fall in the percentage of polymorphonuclear leucocytes. By studying the hydrogen ion concentration one can fairly well predict the cytological picture in the exudate, and *vice versa*. The correlation is evidently very close. In Dogs 7, 2, and to some extent in Dog 10 the pH failed to become acid concomitantly with the progress of the inflammatory reaction. The counts correspondingly reveal a predominance in the percentage of polymorphonuclear cells throughout the period of the experiments (Chart 2, Dog 7).

In Dog 11 the per cent of polymorphonuclears appears surprisingly low for an inflammation of only 24 hours duration; the pH here is 6.98. To summarize, the point under discussion can perhaps be illustrated by the following calculation from Table I. In 31 counts in which the percentage of polymorphonuclears ranged from 60

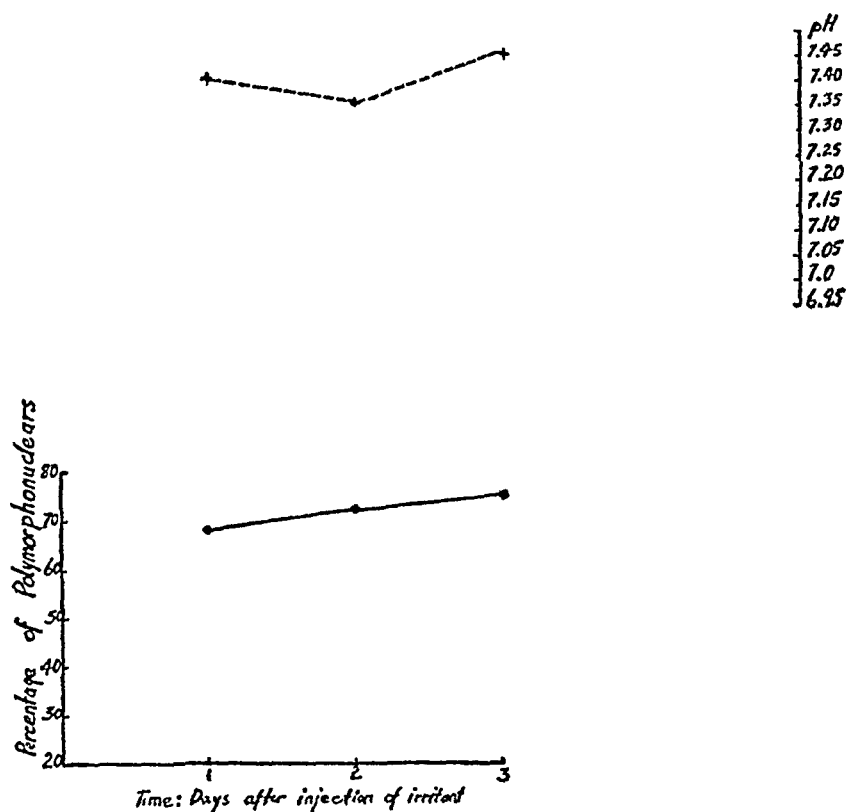


CHART 2

The hydrogen ion concentration in relation to the percentage of polymorphonuclear leucocytes in pleural exudation from Dog 7. Note that the pH remains alkaline and that the percentage of polymorphonuclears maintains a high level throughout the duration of the experiment.

-----+----- pH
 ----- . ----- Percentage of polymorphonuclear leucocytes

to 90 per cent, the pH averaged 7.25. Contrast this with 16 counts in which the percentage of polymorphonuclears ranged from 2 to 60 per cent, and the pH averaged 6.80.

Having obtained definite evidence of the close parallelism between changes in hydrogen ion concentration and in the differential leucocyte formula in acute inflammation the question arose as to which comes first, the changes in the pH or the cellular modifica-

tions. The present data are highly suggestive in answering this question. An examination of the results obtained in the case of some individual experiments points out that the increase in the hydrogen ion concentration evidently precedes the fall in the percentage of polymorphonuclear leucocytes. Chart 3 illustrates this

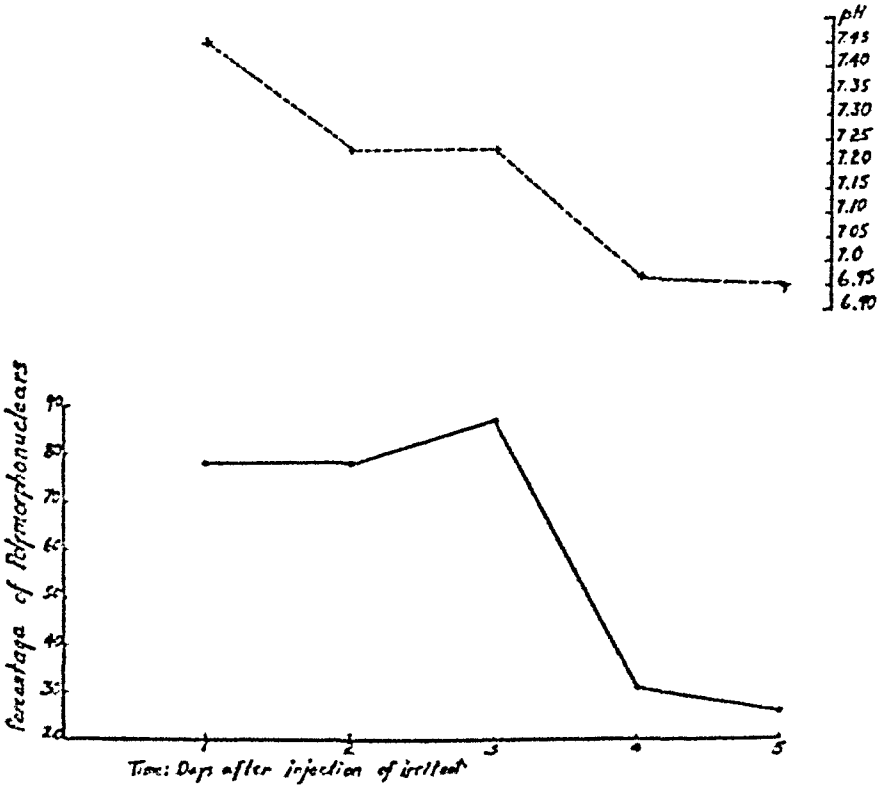


CHART 3

The hydrogen ion concentration in relation to the percentage of polymorphonuclear leucocytes in pleural exudation from Dog 4. Note that the pH steadily declines during the first 2 days, while the percentage of polymorphonuclears remains at a high level.

-----+----- pH
----- . ----- Percentage of polymorphonuclear leucocytes

fact to some extent in the case of Dog 4. Whereas the pH of the exudate steadily declines from an initial value of 7.45, the percentage of polymorphonuclear leucocytes remains high. There was an abrupt fall in the percentage of polymorphonuclears only when the pH reached 6.97. The point is perhaps better exemplified in the case of Dog 13, Chart 4. For the first 2 days the pH was alkaline, 7.4 and 7.5 respectively. The percentage of polymorphonuclears was high, 87.5 and 77.5. On the 3rd day there was an abrupt fall

in pH to 6.98. The percentage of polymorphonuclears, however, was still high, namely 74. On the 4th day the pH was lower than on the preceding day, namely, 6.8. The exudate contained only 10 per cent of polymorphonuclears. Hence in this experiment the sharp rise in hydrogen ion concentration definitely preceded the fall

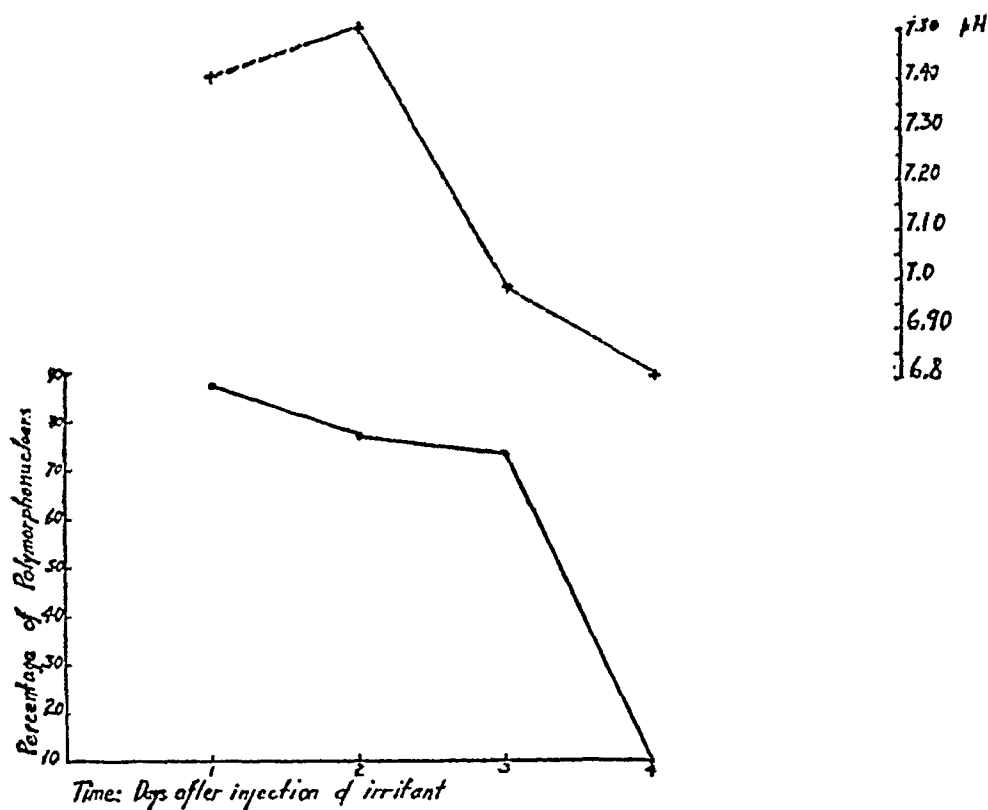


CHART 4

The hydrogen ion concentration in relation to the percentage of polymorphonuclear leucocytes in pleural exudation from Dog 13. Note that the abrupt fall in the pH precedes the sharp drop in the percentage of polymorphonuclear leucocytes.

-----+----- pH
 ————•———— Percentage of polymorphonuclear leucocytes

in the percentage of polymorphonuclear leucocytes. The latter followed the decrease in alkalinity only after the lapse of a definite period. This is definite evidence that the fall in pH precedes the changes in the cytological picture. The hydrogen ion concentration may thus possibly be the regulating factor in determining the differential leucocyte formula of an exudate. In view of what is known of the mechanism of intracellular enzyme action in leucocytes, a physicochemical regulatory mechanism of this type would not be

wholly unexpected. That the fall in pH seems to precede the drop in the percentage of polymorphonuclear leucocytes is quite evident from the above analysis. At the same time it is obvious on examining the data that this relation is not always evident. This seems to depend on the rapidity of the change in reaction. If the rise in hydrogen ion concentration is rapid and sharp the corresponding fall in the percentage of polymorphonuclears may occur so rapidly as to appear to be a parallel phenomenon (see Dog 15, Table I, Chart 5). When the change in reaction proceeds very rapidly the exudate smears invariably reveal numerous degenerated, swollen, and vacuolated polymorphonuclear leucocytes containing characteristically fragmented and intensely stained nuclei. Such lethal effects accompanying an abrupt change in the reaction with increase in the acidity may be an important factor in explaining suppuration at the site of inflammation. In this connection it is perhaps also interesting to note that Rous¹¹ in his studies on factors that determine the reaction of skin grafts came to the conclusion that the developing acidity, in the initial stages at least, when the graft was isolated from its surroundings, was referable to the elements of the tissue proper. (Almost no cells had wandered into the grafts at this time.)

Further experiments were undertaken in an endeavor to modify experimentally the pH of an inflammatory exudate and to determine the effect of such procedure on the differential leucocyte formula. Phosphate buffers (Sørensen) were prepared at pH 6.78 and 7.28. Several cubic centimeters of each of these buffer solutions were injected immediately after the introduction of turpentine into the right chest of dogs. The phosphate buffers were reinjected at intervals of several hours. The periodic withdrawal of pleural exudates showed, however, the same tendency toward an ultimate acidosis and rise in the percentage of mononuclear phagocytes (see Dogs 12, 13, 14, 15, Table I). It became clear that the buffering mechanism of the tissues at the site of inflammation was not easily influenced by the mere introduction of phosphate buffer solutions.

Postmortem examination of the right chest of dogs injected with turpentine several days previously revealed an intense serofibrinous and at times a fibrinopurulent exudate. The pleura was greatly thickened and fibrinous adhesions extended from the visceral to the parietal layers, thus forming small pouches in the pleural cavity. These contained various amounts of exudate. In agreement with

histological studies by Opie⁹ such tissues provided in general the same type of information regarding the cellular infiltration as was obtained from stained exudate smears.

It was of some interest to note that animals which throughout the experiment maintained an alkaline exudate (Dogs 7, 2, and 10)

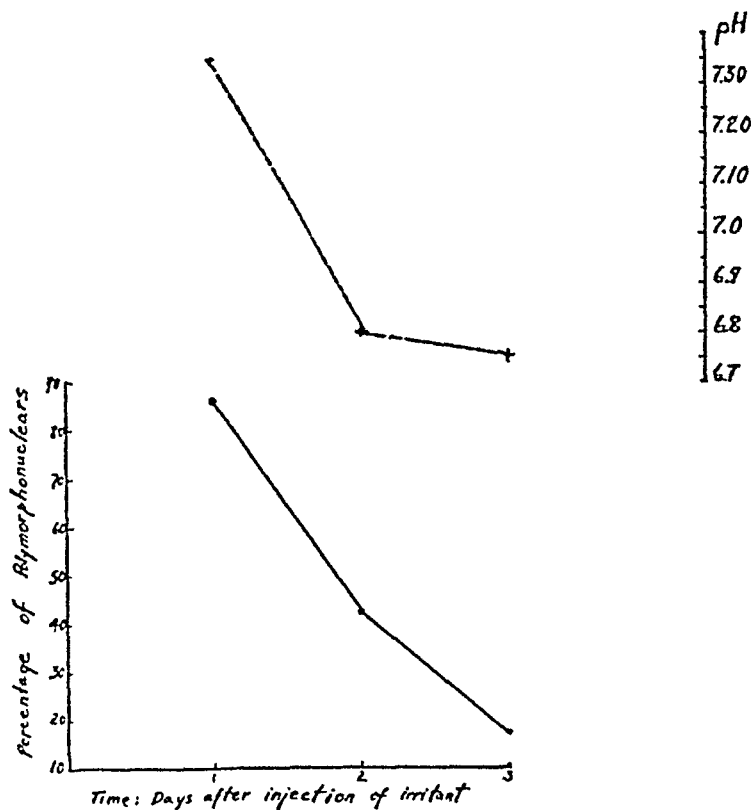


CHART 5

The hydrogen ion concentration in relation to the percentage of polymorphonuclear leucocytes in pleural exudation from Dog 15. Note the abrupt fall in the pH and in the percentage of polymorphonuclear leucocytes on the 2nd day following the injection of the irritant.

-----+----- pH
 -----•----- Percentage of polymorphonuclear leucocytes

appeared in much better physical condition than those whose pleural exudate gradually became acid in reaction. In the latter, dyspnea, weakness and general apathetic behavior were not infrequent.

The effect of reinjection of the same irritant was tried in the case of Dog 2. This animal received 2 cc. of turpentine intrapleurally. Its pleural exudate remained alkaline and showed a high percentage of polymorphonuclear leucocytes for 4 days. The animal was in

perfect condition at the end of the experiment. Thirty-five days later he was reinjected intrapleurally with 2 cc. of the same irritant. Within 2 days the pH was 6.6 and there was an overwhelming number of mononuclear phagocytes in the exudate (Dog 2-A, Table I). The acid pH persisted and on the 3rd day when the experiment was terminated the animal displayed some difficulty in breathing. The pH of the blood was 7.23 and contained 77 per cent of polymorphonuclear leucocytes. In a previous communication the writer¹⁴ pointed out that an area of inflammation is ultimately walled off from the rest of the organism; the inflamed area develops its own local circulation, its own hydrogen ion concentration and its own metabolism. This view is substantiated when the data on the pH of the blood are compared with those obtained in the majority of exudates in the later stages of the inflammatory reaction (Table I).

DISCUSSION

The results obtained in this series of experiments reveal the fact that in most instances an acute pleural inflammation induced by a strong chemical irritant such as turpentine gradually develops a local acidosis. Furthermore, the data point toward a definite relation between the hydrogen ion concentration and the cytological picture of an inflammatory exudate. The findings certainly indicate the occurrence of a parallel between the hydrogen ion concentration of the fluid medium and that favorable to the action of the enzyme of the predominating phagocyte. The relation between the two phenomena is strongly suggested by the following considerations.

In the first place, although inflammation as such conduces to local acidosis with a concomitant shift in the cell counts from polymorphonuclear to mononuclear cells, it is interesting to note that in several experiments an alkalinity was maintained throughout the period of the inflammation (see Dogs 7, 2, 10, Table I). In such cases, and only under these conditions, was there no shift in the cell counts, the percentage of polymorphonuclears remaining at a constantly high level even in the later stages of the inflammatory process. It becomes somewhat difficult to consider this state of affairs mere coincidence. Secondly, the fact that by determining the pH of the exudate the character of the cytological picture could be

fairly well predicted and *vice versa* seems to be definite evidence of some correlation between the pH and cell count. In the third place, the fact that in a few instances when the shift from alkaline to acid took place rapidly the cell change, although delayed, nevertheless invariably followed appears to warrant the inference that if there is an interdependence it is the pH that conditions the cytological picture and not the reverse order of sequence. The observations reported in this communication seem therefore to support the conclusion that the differential leucocyte picture at a given time in the development of an inflammatory reaction is a function of the pH of the exudate.

The implications of this concept are obvious. It is possible that an understanding of the histological differences of various inflammatory lesions may be facilitated through a study of their respective hydrogen ion concentrations.

Opie⁵ pointed out that the studies on intracellular enzymes of leucocytes have served to explain many of the phenomena of resolution. Some of his earlier conclusions¹⁵ on the solution of tissue with abscess deserve perhaps revision, in view of the present observations. Briefly stated, Opie's original experiments on abscess formation consisted in inducing a purulent exudation by the subcutaneous injection of turpentine. Four or 5 days later a large cavity distended with fairly thick purulent fluid was formed. The cells of this pus were separated from the serum by centrifugalization. To the cell-free pus serum, leucoprotease was added. This combination freely digested coagulated serum. On the other hand, the same polymorphonuclear enzyme in the presence of blood serum failed to digest materially the coagulated serum. From these facts Opie concluded that the anti-enzymatic action of a limited quantity of exuded serum is overcome by an increasing quantity of proteolytic enzyme set free by disintegration of polymorphonuclear leucocytes, thus accounting for the solvent effect on tissues of a purulent exudation. This conclusion is now perhaps somewhat difficult to accept; at least the data presented in this paper open the way to a different interpretation. It has been found (Table I) that as a rule 4 or 5 days following the onset of an inflammatory process induced by turpentine the resulting purulent exudate is usually characterized by an acid reaction. Opie has demonstrated that both leucoprotease and anti-enzymes are inactive in an acid medium. It is therefore

more likely that the solution of fibrin or necrotic tissue in a purulent area of inflammation is due to the activation of autolytic enzymes by the acid reaction. Enzymes of this type have been adequately described in a recent review by Bradley.¹⁶ This interpretation appears to be more in accord with the facts since it is hardly possible to assume in view of Opie's own findings that an excess of leucoprotease from disintegrating polymorphonuclears could act in an acid medium. It seems probable that in Opie's experiment the proteolytic enzyme, leucoprotease, was inactivated by the acid cell-free pus, while at the same time there were present in this fluid autolytic tissue enzymes that possessed an optimum activity in an acid pH and were hence able to digest the coagulated serum. Opie's earlier view on the mechanism of caseation, which he considered to be the result of an accumulation of autolytic enzymes released from epithelioid cells and which acted in an approximately neutral or weakly acid medium, is not wholly dissimilar to the writer's view, as just expressed in regard to the solution of fibrin and necrotic tissues in an abscess.¹⁷ Furthermore, it is to be noted that Opie himself pointed out in some of his later studies⁹ that whereas leucoprotease may play a part early in the digestion of fibrin, the latter undergoes solution in the advanced stages of an inflammatory reaction only in the presence of weak acid. Opie^{5,9} therefore concluded that fibrin is ultimately digested by an enzyme having the character of lymphoprotease and resembling the autolytic enzymes of tissues. In an endeavor to throw further light on the question under discussion 1.5 cc. of turpentine were injected subcutaneously into the right flank of a dog. Four days later a large subcutaneous abscess containing thick viscous pus resulted. The pH of this exudative material was definitely acid in reaction, approximately 6.6. This would support the contention that an excess of leucoprotease could not possibly be the important factor in the solution of tissues in such abscesses since this enzyme is active only in an alkaline or neutral medium.

Bayliss,¹⁸ as a result of his studies on emulsin, expressed considerable doubt as to the actual existence of true anti-enzymes as follows: "Some of the effects described as being due to them are to be accounted for by changes of hydrogen ion concentration, others to adsorption of the enzyme by a colloid." Bayliss pointed out that in his emulsin experiments the effect was found to be due merely to

diminution of the acidity of the solution. The inhibitory effect of the anti-enzyme disappeared when the solution was brought back to the initial value by the addition of acid phosphate. This idea may doubtless have considerable importance in revising our accepted concepts concerning so-called "anti-enzymes" in inflammation, especially in view of the progressive increase in hydrogen ion concentration in such pathological areas. Nevertheless, although Bayliss may be correct as far as anti-enzymes are not comparable in regard to specificity to the true antibody, still by counteracting the effectiveness of the enzyme, whether by adsorption or by changes in the hydrogen ion concentration, the anti-enzymatic effect of serum on enzymes remains a fact.

The mechanism conducing to local acidosis in inflammation is still somewhat problematical. Schade and his co-workers¹⁹ reported that pus from acute abscesses had a pH ranging from 5.95 to 6.50; the pH of normal tissue fluids ranging from about pH 7.10 to pH 7.40. The studies of Irisawa²⁰ and of Ito²¹ have shown that lactic acid is a constant constituent of pus. Gessler²² has demonstrated that the oxygen consumption and the metabolic rate are increased in an inflamed area. This state of affairs would doubtless favor the development of a local acidosis unless properly compensated by an equally increased and effective fluid circulation at the site of inflammation. The writer has shown in previous studies that various foreign substances, including bacteria and electrolytes, are unable to escape readily from the site of inflammation owing to the presence of a fibrinous network and of thrombosed lymphatics. Furthermore, in acutely inflamed areas of moderately long standing a number of vascular capillaries have also been found with their lumina occluded by thrombi.³² It is conceivable, therefore, that as acid metabolites are formed in an acutely inflamed area these tend to be fixed *in situ*, thus causing a rise in the hydrogen ion concentration of the exudate.

SUMMARY AND CONCLUSIONS

A pleural inflammatory exudate, in the majority of instances, develops a rise in its hydrogen ion concentration concomitantly with the progress of the inflammatory reaction.

When the pH of the exudate is alkaline the percentage of poly-

morphonuclears at the site of inflammation exceeds that of the mononuclear phagocytic cells.

When the pH of the exudate is approximately neutral the percentage of polymorphonuclear cells tends to approach that of the mononuclear phagocytes.

When the pH of the exudate is definitely acid large numbers of polymorphonuclear cells are found degenerated. The percentage of relatively normal appearing polymorphonuclear leucocytes is found considerably lower than that of the mononuclear phagocytes.

In some cases the pH of the exudate remains alkaline throughout the period of an acute pleural inflammation. In these instances the percentage of polymorphonuclears invariably exceeds that of the mononuclears.

By measuring the hydrogen ion concentration of an inflammatory exudate the character of the cytological picture can be predicted with a fair degree of certainty. Likewise the converse follows.

Evidence has been obtained to show that the development of a local acidosis in an area of inflammation precedes at times the changes occurring in the differential leucocyte formula of the exudate. In such cases, however, the cytological changes ultimately follow the development of the acid reaction.

The observations reported suggest that the differential leucocyte formula in an area of acute inflammation is a function of the hydrogen ion concentration of the exudate. The cytological picture in an inflamed area seems to be conditioned by the pH of the exudate surrounding the injured tissue. The present study indicates that the developing local acidosis as the inflammatory reaction progresses can adequately account for the shift in infiltration from polymorphonuclear leucocytes to mononuclear phagocytes at the site of inflammation.

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THE CULTIVATION OF MEXICAN AND EUROPEAN TYPHUS RICKETTSIAE IN THE CHORIO-ALLANTOIC MEMBRANE OF THE CHICK EMBRYO *

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In the course of studies on typhus fever continued in this laboratory efforts have been made to compare the biological and serological properties of the Mexican and the European virus strains. While the two are beyond question closely related, determinable immunological differences have recently and clearly been brought out in the vaccination and passive immunization experiments and in the serological reactions described by Zinsser and Castaneda.¹ The most troublesome difference, however, has been the fact that it has not been possible to obtain as extraordinary an accumulation of *Rickettsiae* with the European strain by the rat X-ray method as was possible with the Mexican strain, as a practical method in vaccine production. In attempting to gain more insight into the existing differences a number of experiments have been carried out in this laboratory, the chief purpose of which was to study the two varieties of *Rickettsiae* against the same biological background, other than the louse intestine in which they appear and behave entirely alike. The following experiments would not have been performed had it not been for a casual visit to this laboratory of Dr. Ernest Goodpasture, who described to us in detail his cultivation of a variety of ultramicroscopic agents by the "fertile egg" method, details of which have since appeared in a number of publications from his department.^{2, 3, 4} We take this opportunity of listing him, in this manner, as a co-author. We made no changes in the technique that he described, except in point of time and temperature of incubation — matters deemed advisable in view of the experience with *Rickettsiae* gained here. The technique in brief, then, is as follows.

* Received for publication September 15, 1933.

METHOD

Fertile hen's eggs were incubated at 37.5° to 38° C for 8 to 9 days. At the end of this time the eggs were washed with alcohol and flamed. Windows 0.5 to 1 cm. square were cut in the shell by means of a razor blade. In most instances the inside acellular shell membrane was not injured. Hot paraffin was allowed to flow over this layer, which was then opened by cutting around the edges of the window with a pair of fine scissors. A small amount of emulsified tunica

TABLE I

Summary of Results of Cultivation of Rickettsiae in the Chorio-Allantoic Membrane of the Chick Embryo

Strain	Inoculum	Total number of eggs inoculated	No. of eggs with dead embryos	No. of eggs with living embryo	
				Positive for <i>Rickettsiae</i>	Negative for <i>Rickettsiae</i>
Mexican <i>Rickettsiae</i>	Tunica	23	13	4	6
	Spleen	10	1	1*	8
	Egg	15	9	4*	2
European <i>Rickettsiae</i>	Brain	13	9	0	4
	Spleen	12	7	2	3
	Brain and spleen	9	7	1	1

* One each by smears only.

exudate or brain and spleen material was dropped with a capillary pipette on the extra-embryonic membrane, the chorion being uppermost. A sterile coverslip was placed over the opening and it was sealed with hot paraffin. The eggs were then reincubated at 33° C and opened after 7 to 10 days for examination. Smears were stained with Castaneda's methylene blue-safranin stain,⁶ and tissue fixed in Regaud's solution (potassium bichromate 2.5 gm., sodium sulphate 1 gm., water 100 cc., to which is added 20 cc. of formalin immediately before use) and later stained by Giemsa's method. In several instances the material was also inoculated into guinea pigs and a typical response was obtained in these animals. Immunity tests showed them to be protected from subsequent homologous infections. Cultures for bacteria were made from the eggs in which no obvious signs of contamination could be observed, and in only four instances was there any growth on blood agar plates.

RESULTS

The results of these experiments are summarized in Table I. In general it was easier to infect eggs with Mexican *Rickettsiae* and these appeared greater in number. Many embryos were found dead a few days after inoculation. This was particularly true with the European *Rickettsiae*. Even discounting the eggs with dead or autolyzed embryos, in which we never found positive results, the percentage of positive findings was so low and the amount of virus obtained so scarce that it was impossible to make vaccine from them. However, the microscopic appearance of the infected chorio-allantoic membrane seems to be of sufficient additional interest and this is, therefore, briefly described.

DETAILS OF REPRESENTATIVE EXPERIMENTS

1. *Experiments with Mexican Rickettsiae*: Eggs were received October 21, 1932, and kept at 37.5° C until October 31st. On this day three eggs inoculated with emulsified tunica material from Guinea pig 365 showed typical lesions with *Rickettsiae*.

Egg 1 kept at 33° C and opened November 3rd was negative. Egg 2 kept at 38° C and opened November 4th was negative. Egg 3 kept at 33° C and opened November 8th showed a thickened membrane over the exposed area, not adherent to the shell, but there were no signs of bacterial infection. The embryo was alive. A smear showed but few *Rickettsiae*. Part of the membrane was inoculated into Guinea pig 372, which showed slight fever but typical tunica swelling. The same animal inoculated again with tunica material from a Mexican typhus guinea pig showed complete immunity. Cultures from the egg on blood agar plates were sterile. The main part of the infected membrane was fixed in Regaud's solution and sections were cut and stained with Giemsa. Figure 1 is a drawing made from a microscopic field of this membrane under a magnification of 600 diameters. It will be seen that there are *Rickettsiae* crowded in some of the cells of the ectodermal layer which, in morphology, staining reaction with Giemsa, and in their intracellular positions, are indistinguishable from the same organisms seen in the tunica cells of guinea pigs or in the intestinal cells of human lice, polyplax or fleas.

2. *Experiments with European Rickettsiae*: Eggs were received November 18th and kept at 38° C. Three eggs were inoculated on November 22nd with material from Guinea pig 377, suffering at the time from a typical attack of European typhus infection. The temperature of the guinea pig was 106° F when it was sacrificed, 9 days after intraperitoneal inoculation. There was no scrotal swelling at the time.

Egg 1 was inoculated with brain, Egg 2 with brain and spleen, and Egg 3 with spleen only. It is important to note that, as usual, no *Rickettsiae* could be found in the tissue material inoculated.

On November 29th Eggs 1 and 2 were opened. In Egg 1 we had negative results. There apparently was no change — the membranes were not thickened and smears and sections were both negative for *Rickettsiae*. Egg 2, inoculated with brain and spleen, showed slight thickening of the membrane, and on smear a few intracellular and extracellular *Rickettsiae* were found after prolonged search. This membrane was cultured on blood agar with negative results. Sections fixed in Regaud's solution and stained with Giemsa showed a fairly large number of cells containing typical *Rickettsiae*.

Egg 3 opened on December 2nd was negative. Figure 2 represents a drawing of cells containing the organisms. In morphology, in staining reaction to Giemsa and in intracellular grouping these organisms were identical with *Rickettsiae* as seen in the cells of louse intestine infected with European typhus, and appeared to be identical with the Mexican *Rickettsiae* in the other eggs, except that perhaps they were in average measurement slightly smaller.

A part of the membrane from Egg 2 was inoculated into Guinea pig 390. This animal showed a typical temperature curve, reaching 105° F and above on the 8th and 9th day, rising to 106° F on the 10th and 11th day, and on the 12th day, when the temperature was 105.5° F, the animal was killed for histological examination and for inoculation. Guinea pigs 399 and 400 were inoculated with brain and blood from this animal and later both showed typical passage strain reactions. The brains of both Guinea pigs 399 and 399 showed characteristic brain lesions in considerable profusion. These have been independently checked by a number of experienced observers. The absence of scrotal swelling, indeed, as well as the profusion of the brain lesions, characterizes it without doubt to be of the European type of infection. This is further borne out by

the immunity test. Guinea pigs of the third, fourth, and fifth generations (Nos. 414, 418 and 443) were reinoculated, after 6 weeks to 2 months, with material from European typhus guinea pigs, and all three were completely immune.

3. *Experiments with Subcultures in Eggs:* We were interested to see if the amount of *Rickettsiae* in these membranes might not be increased by repeated transfer from egg to egg. One of these experiments is as follows.

On January 19th four eggs were inoculated with tunica material from Mexican typhus Guinea pig 447, smears from which showed *Rickettsiae*. Egg 1 opened January 28th showed a living embryo with a thickened membrane. Blood agar plate cultures were sterile. Both smear and section were positive for *Rickettsiae*. The material was ground in a mortar and inoculated into four new eggs. Among these, Eggs 6 and 7 were autolyzed, the other two showing positive results. Material from one of these, Egg 8, was inoculated into another series of four eggs, with positive results in one. While there was suggestive evidence of an increase in the number of *Rickettsiae* in the eggs of the second and third generations, this did not appear to be of sufficient degree to warrant further transfer.

HISTOLOGICAL EXAMINATION

Changes Observed 8 to 9 Days after Infection: The appearance of the normal chorio-allantoic membrane from hatching chicks has been described by Woodruff and Goodpasture.² It consists of a thin layer of reticulated mesothelial tissue lined by one or two celled layers of ectodermal and endodermal tissue. At this stage of development the ectodermal layer is often absent. A great change occurs when infection with *Rickettsiae* takes place. The whole membrane with all three layers is much thickened. This naturally varies with the degree of infection. In the lightly infected membranes solitary thickening of only the ectodermal lining takes place. Usually, however, the number of cellular elements in the mesothelial layer is found to be increased. In a case of average severity the ectodermal lining is about ten cells thick, covered on the outside with a layer of degenerated cells. The endodermal lining is slightly thickened. There is a great increase in the cells with a deeply stained, round nucleus in the mesothelial

layer. These often form clumps or nodules of various sizes. Most of the cellular elements in these nodules are far too degenerated to be differentiated. A large number of cells containing coarse eosinophilic granules also appear grouped with the mononuclear cells. Often in the center of these nodules small blood vessels containing red blood cells can be distinguished. Figure 3 is a photomicrograph showing one of these nodules, together with the increased cellular element in the surrounding areas. It is interesting to note that typhus infection of the chorio-allantoic membrane gives an entirely different picture from that infected with vaccinia or fowl pox. In fact, when the organisms are few the presence of these changes often encourages prolonged search and frequently leads to subsequent finding of the organisms.

Distribution of Organisms in the Infected Chorio-Allantoic Membrane: By far the majority of the *Rickettsiae* are found in the degenerated outer layer of the ectodermal lining. There they are mostly intracellular and often in large clumps. A few are also found in the tissue spaces and these often assume a much elongated form. Pinkerton⁶ considers these the most actively growing *Rickettsiae*, as shown in his tissue culture experiments. When the infection is heavy a few *Rickettsiae* may be found in some of these nodules. How they reach there it is difficult to determine. In one or two sections we have observed them along the wall of a small capillary, but we have not succeeded in demonstrating them in the endothelial cells lining these vessels. One may perhaps speculate about the formation of these nodules as starting from tissue reaction to local deposit of organisms and their metabolic products, with subsequent death of these cells due to impairment of blood supply.

DISCUSSION

While few students of typhus fever have any doubts concerning the etiological importance of *Rickettsiae*, both in the European and the Mexican infections, there still arise occasional questions regarding the significance of the organisms found by Mooser in the tunica lesions. While we believe that the work of Mooser, as well as the extensive experimental cross-indexing of the facts bearing upon this point undertaken in this laboratory, has removed all possibility of error, every additional point of evidence is of value in so important a question.

The fact that the eggs inoculated with the Mexican tunica material develop morphologically typical *Rickettsiae* and that attempts to cultivate bacteria from these eggs are unsuccessful, added to the characteristic results of inoculation of the egg material into animals, brings further evidence to the array of proof already submitted.

In regard to the inoculations of the eggs with European material the results indicate that from tissue material in which *Rickettsiae* are apparently too few to be found by smear a culture can be produced in which they are plentifully apparent and from which the disease can again be propagated. The similarity of these cultivated *Rickettsiae* to those found in the European lice adds, we think, more strength to the assumption of the etiological significance of the *Rickettsia prowazeki*.

Furthermore, it has been of exceptional interest to us that by identical methods the European and the Mexican *Rickettsiae* can be studied against the same biological background and proved to be indistinguishable in their behavior under the same cultural conditions. This is a direct demonstration of the close similarity between the two organisms and should assist materially in removing any lingering doubt as to whether Mooser's organism may have been picked up in experimental animals during inoculation passage or not. This question was raised not long ago and every point of evidence that can clarify it is of more than ordinary importance because of the extensive endeavors to produce prophylactic vaccines and potent antityphus sera with the Mexican tunica organisms.

The fact that the inoculation of guinea pigs from the European and Mexican typhus infected eggs, respectively, has produced the two characteristic types of the disease is another point of evidence that, in spite of their close similarities, the two organisms are not absolutely identical. Though possibly derived from the same original stock, adaptation in passage through rodents, fleas and polyplax may be the cause of slight biological modifications in the Mexican strain.

Our results in the egg method of cultivation so far have not given much hope that we may obtain a sufficient number of *Rickettsiae* in this way. Several egg to egg transfers have not added materially to the yield. However, the suggestive evidence of these interesting histological appearances may perhaps throw some light on the formation of the typhus nodules in man and in experimental animals.

SUMMARY

It has been found that both Mexican and European typhus *Rickettsiae* are able to infect the chorio-allantoic membrane of the chick embryo, although the results do not lead us to hope for its practical use in the production of vaccines. The interesting histological appearance of the typhus-infected membrane and the distribution of *Rickettsiae* are briefly described. Possible significance of this finding to clarify further the relation between *Rickettsia prowazeki* and Mooser's bodies is discussed.

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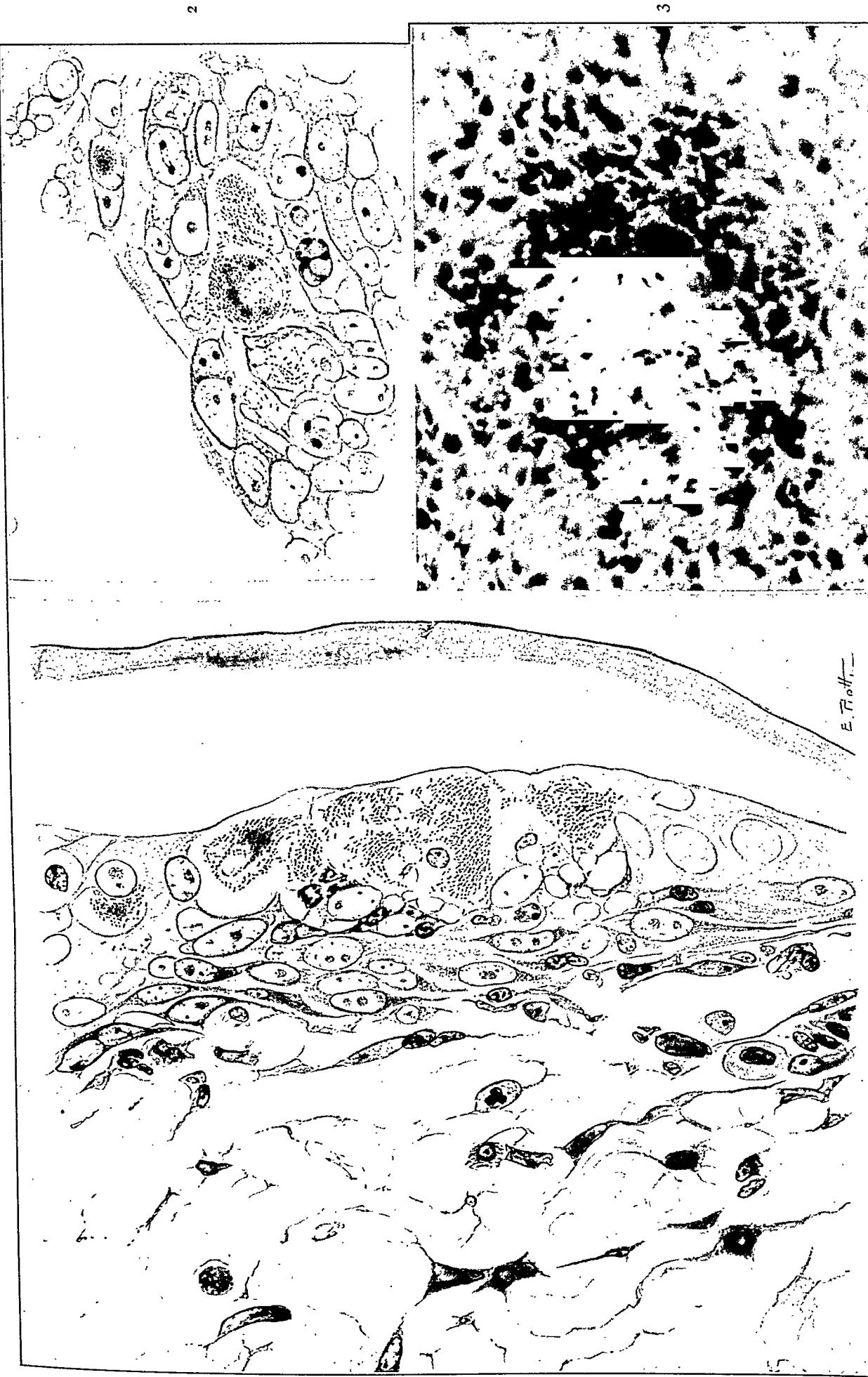
DESCRIPTION OF PLATE

PLATE 70

FIG. 1. Drawing of a Giemsa-stained paraffin section of egg inoculated with tunica material from a Mexican typhus guinea pig showing *Rickettsiae* of the Mooser type.

FIG. 2. Drawing of a Giemsa-stained paraffin section of embryonic membrane from an egg inoculated with spleen and brain tissue from a guinea pig infected with European typhus of the Breinl strain, and showing *Rickettsiae* *prowazeki*.

FIG. 3. Photomicrograph showing one of the nodules in the mesothelial layer. $\times 620$.



CYTOPATHOLOGICAL STUDIES OF MORPHINE POISONING AND
CHRONIC MORPHINISM IN THE ALBINO RAT,
WITH REFERENCE TO SUBSEQUENT
LECITHIN TREATMENT *

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I. INTRODUCTION

Recently Wen Chao Ma ¹ has described in detail the cytopathology of acute and chronic morphinism in the albino rat, as well as suggested the importance of lecithin administration as a therapeutic agent during recovery from chronic morphine poisoning. Apart from reporting the pathological action of morphine upon the tissues as a whole, this author made a detailed, systematic cytologi-

* Aided by a grant made by the Rockefeller Foundation to Washington University for research in science.

Received for publication September 25, 1933.

cal investigation of the behavior of the cell inclusions during temporary and prolonged treatment. These observations led him to conclude that by studying their consistent and distinctive reactions to the drug during the various phases of poisoning he was able to employ both the Golgi apparatus and the mitochondria as indicators of physiological depression under pathological conditions.

Bearing in mind the recent controversies concerning the functional significance of these cell organoids the contentions of Ma are of importance. Therefore, in view of these significant results and partly because this problem covers certain ground in which there is, apparently, some misconception, it was deemed advisable to re-investigate this work, not only by applying more recent cytological procedures but also by including additional experiments that might lead to more conclusive results.*

II. MATERIAL AND TECHNIQUES

Albino rats of both sexes, segregated shortly after weaning, belonging to the same strain and weighing approximately 195 to 215 gm., were selected for the study of acute and chronic morphinism, while animals from the same litters were isolated and kept as controls. Subcutaneous injections of morphine hydrochloride administered hypodermically were used throughout the experiments, and in order to induce acute poisoning each rodent received a single dose of 5 cc. of a 2 per cent solution (10 mg. of morphine). The rats set aside to become addicts were first given an inoculation of 3 mg. as a 1 per cent solution in water, the strength of which was increased by 1 mg. every 10 days. After a period of 7 months the daily doses of morphine were discontinued.

Tissues were obtained and fixed from those rats under acute morphinism, at intervals ranging from 1 to 24 hours after injection. In the addicts material was taken at various periods (as indicated in the text), both before and after the daily injections of the drug were discontinued.

The lecithin used was prepared by Merck from eggs; 1 gm. was mixed with 40 gm. of ordinary rat diet of vegetables and bran. A second group of rodents, of precisely the same weight and age as the first, was isolated and fed for a period of 7 months on 4½ gm. of lecithin mixed with 20 gm. of regular food, and given to the animals in the ordinary way.

Glandular Tissues: The glandular tissues studied in this investigation consisted of liver, pancreas, thyroid, submaxillary gland, kidney, stomach and intestine.

Nervous Tissues: The nervous tissues observed were spinal ganglia, Purkinje cells of the cerebellum, motor cells of the spinal cord, as well as the large pyramidal neurones of the cerebral cortex.

* I wish to take this opportunity of thanking Dr. E. V. Cowdry, not only for his kind hospitality, but also for his encouraging interest in this investigation.

Muscle Tissue: The smooth muscular coats of the intestine, skeletal and cardiac muscles were employed.

General Cytological Preparations: For general cytological preparations the tissues were cut into very small pieces in order to ensure rapid penetration of the reagents. Overnight fixation in ice cold Flemming's solution was used, as recommended by Ludford,² omitting the 0.5 per cent urea. Sections were stained in iron alum hematoxylin following bleaching with hydrogen peroxide.

Mitochondria: Tissues were treated by Regaud's fixative, in which the sodium sulphate was omitted, and then stained with acid fuchsin and methyl green. This method gave excellent results for all tissues other than brain and spinal cord, for which the Champy-Kull anilin fuchsin technique is recommended. Flemming's solution without acetic acid, followed by staining with Heidenhain's hematoxylin, together with the modifications mentioned by Ma, Lim and Liu³ was also employed.

Golgi Apparatus: The most successful results were obtained by Ludford's variation of Nasonov's Golgi technique. Instead of staining according to the Volkonsky method, orange G in 95 per cent alcohol was found to give preferable results. In some cases the Nasonov preparations were stained by the Kull method, using toluidin blue and anilin fuchsin. Dehydrating and embedding were always completed in one day, and excellent preparations were obtained by using carbon di-sulphide as a clearing agent. Experiments in times of fixation showed that brain, spinal cord, pancreas and liver render better Golgi bodies after a prolonged fixation of 36 hours instead of the prescribed 24, followed by osmication up to 5½ days at 35° C. Hirschler's modification (1918) was also employed, but the results obtained were more inconsistent than those of the above method. Da Fano's cobalt nitrate (formalin 15 per cent) and Cajal's uranium nitrate techniques were occasionally used. When preparing the reducing fluids for these silver techniques the sodium sulphite was excluded, and the times of fixation for gland tissues were reduced to 3 to 4 hours. Brain and spinal cord, on the other hand, were fixed for 8 to 10 hours.

For studying the mineral organization of the normal and experimental tissues the microincineration procedure, by which the protein compounds of the cells are burned out leaving behind only the mineral residue, was used. The method is similar to that recently described by Policard.⁴ Small pieces of tissue were fixed in a solution of 9 volumes of absolute alcohol to 1 volume of neutral formalin. After fixation for 24 hours the material was passed through several stages of absolute alcohol to ensure a complete dehydration and also to remove the formalin. The material was embedded in the usual manner and sectioned at 5 microns. The preparations were then placed on a small quartz slab and transferred to a special electric quartz oven and incinerated at temperatures varying from 625° to 650° C, for periods of 25 to 45 minutes.

The arrangement and deposition of the mineral constituents in the incinerated sections were studied in dark-field illumination obtained by using a Zeiss cardioid condenser. A large sized Spencer Mazda microscope lamp, catalogued as number 394 and equipped with a 500 watt Mazda gas-filled concentrated filament bulb, was used. Directly in front of the bulb was a pair of 4½ inch condensing lenses, which threw a converging beam of light through the cone, illuminating an area about 2 inches in diameter. A pale blue frosted glass, inserted in front of the cone and placed 4½ inches from the substage mirror of the microscope, gave the best illumination for observing the mineral distribution in the incinerated tissues.

The Altmann technique for fixation by drying while freezing, as recently elaborated by Gersh,⁵ was also employed for studying the pancreas and liver.* As time would not permit, only normal material and tissue from a rat that had previously received the prescribed single large dose of morphine † were examined by this method $5\frac{1}{2}$ hours after administration of the drug. This procedure consists mainly in freezing the tissue quickly by liquid air prior to dehydration *in vacuo* at -20°C . After embedding in paraffin the material was mounted dry, sectioned at 8 microns and stained by Heidenhain's iron hematoxylin and counterstained in eosin. Tissues thus treated stain extremely rapidly, it being necessary to leave them in the hematoxylin only for 30 to 60 seconds. The advantages of this technique, apart from the rapidity of fixation, are that it should eliminate the possibility of shrinkage, for Gersh contends that when a portion of tissue is maintained at a temperature of -20°C , and dehydrated by means of the Altmann apparatus, the volume of the block of tissue remains unchanged after the process.

III. ON THE FUNCTIONAL SIGNIFICANCE OF THE CELL ORGANS AND THEIR BEHAVIOR DURING CELL INJURY AND DISEASE

Before the effects of morphine poisoning upon the general cytology of the cell can be interpreted it will be well to discuss briefly the possible functional significance of the several cell structures under normal and abnormal conditions.

A review of the literature dealing with the physicochemical nature of mitochondria shows that an enzymatic conception of mitochondrial activity is gradually being realized.⁶⁻⁷⁻⁸ This outlook, from a chemical point of view, is established largely on the investigations of Marston⁹ and his co-workers, who conclude, on the basis of their reaction to azine dyestuffs, that mitochondria contain proteolytic enzymes. In addition to this, Robertson,¹⁰ who has carried out an independent study on the function of the lipoid in the mitochondria, has shown that at the phase boundary of the mitochondria and protoplasm synthesis by enzymes may occur. These conclusions derived from the chemical study of the problem receive support when correlated with certain cytological evidence. The assertion of Cowdry,¹¹ wherein the phase boundary and the surrounding protoplasm of mitochondria are regarded as the seat of processes of

* I wish to express my gratitude to Prof. R. R. Bensley for his hospitality during my visit to his laboratory, and also to Dr. Gersh for kindly fixing the above-mentioned tissues.

† As morphine hydrochloride was not available for this experiment, morphine sulphate was substituted. As far as is known there is no difference in chemical action.

elaboration, commencing with the absorption of molecules of certain solutes, and ending in a series of physical and chemical interactions between the mitochondria and the incoming substances, is of interest, as this would naturally lead to the production of new compounds of widely different natures. These theories explain, to a large extent, the behavior of mitochondria within the animal and plant cells, where they appear to be closely associated with the production of cellular materials.¹²⁻¹⁵

Another school of thought, however, brings forward evidence to show that the Golgi apparatus alone is associated with secretory processes, and that during this phenomenon the mitochondria play no active rôle. There seems little doubt that the process of fat absorption in the mammalian intestine is correlated directly with the Golgi apparatus.¹⁶ During secretion in the thyroid gland it has been conclusively shown that both the Golgi bodies and the mitochondria are engaged in the process of secretion.¹⁷

Ma, Lim and Liu,¹⁸ on the other hand, have taken an independent course and conclude that "the Golgi material is the lipoid or fatty component of the mitochondria, which becomes demonstrable on the dissociation of the mitochondrial substance."

Ludford,¹⁹ assuming a broader view of the problem, has taken into consideration the function of the Golgi bodies and correlated it with an enzymatic conception of mitochondrial activity and concludes that synthesis by enzymes may occur at the protoplasmic interface. The resulting products continually diffuse into the cytoplasm, thereby preventing an accumulation at the surface of the mitochondrion, which would inhibit further synthesis. He suggests that these elaborated cell products are concentrated into droplets at the surface of the Golgi apparatus prior to their elimination.

Ludford's theory of a functional interrelation existing between the Golgi apparatus and the mitochondria furnishes an explanation for the behavior of these structures during cellular metabolism, and deserves serious consideration.

Now that the function of these bodies has been considered during normal metabolism it will be necessary to review briefly their response to abnormal conditions and disease. The interesting question arises, and incidentally is still a matter of debate, as to whether or not the reaction of cell inclusions to pathological conditions can be accepted as a diagnostic criterion of disease.

Earlier authors, among whom were Tello,²⁰ Fananás²¹ and Legendre,²² first reported a definite alteration and behavior of the Golgi apparatus during malignancy, while several others have arrived at contrary conclusions. The general literature in this field is far too conflicting to warrant any detailed discussion, but nevertheless some of the more recent work in relation to the response of these cell organs, following cellular injury and to the administration of chemicals, is of extreme importance when considering the cytopathological action of morphine. In this field experiments of Policard, Garnier and Scott are of interest. The former authors reported that the administration of phlorizin causes a notable decrease in the amount of mitochondrial substance within the tubules of the kidney.²³ Scott,²⁴ when observing the effect of phosphorous poisoning upon the mitochondria in the acinar cells of the guinea pig, found that these inclusions respond by eventually breaking down into fat granules.

The association of clinical symptoms with functional behavior of the cell organs in severe pathological conditions has been reported. Goetsch²⁵ has observed a marked increase in mitochondria in many cases of hyperthyroidism, and Homans²⁶ has correlated mitochondrial changes in the β cells of the islets of Langerhans with diabetic lesions.

The recent work of Ludford²⁷ on the biological action of X-rays on malignant growths also shows that cytological changes are induced by irradiation. In certain types of transplantable tumors he found that irradiation results in an immediate action on the mitochondria (which become vesicular within 40 minutes), while the Golgi apparatus responds by hypertrophy and fragmentation. Moreover, it is reported that after irradiation enlargement of the cells is accompanied by a definite increase in the mitochondria and an enlargement of the Golgi material.

The physiological investigations of Hall and MacKay,⁷ on the effect of administration of glucose on liver cells, show that glucose causes hypertrophy and enspherulation of mitochondria. By these studies they have established a relation between the mitochondria of the hepatic cells and the glucose-glycogen equilibrium. Further evidence is afforded by Weatherford,²⁸ who, while observing mitochondrial changes in the initial stages of acute inflammation of connective tissue cells, has shown that they give a definite morphological re-

action to this condition and form chainlets, which finally segment into spherules, thus indicating their sensitivity to cellular injury. Nahm²⁹ has likewise lately shown that the Golgi elements are more readily demonstrated in certain tissues after short autolysis than in the normal.

Although the general literature concerning the changes of these cytological constituents in cell injury and pathological conditions is somewhat conflicting, the evidence as a whole indicates that they are responsive, but at the same time inconsistent in their morphological reactions to such changes. Assuming these cell organs to be the seat of enzymatic action within the living cell, the differences of opinion concerning their behavior under varying conditions might possibly be due to the fact that observations were made during different phases of cellular functioning when their morphological and staining reactions would alter accordingly.

Various authors, when writing of morphine tolerance, point out that there is no substance in the body that will neutralize the poison. Consequently it is natural that one should look for some direct change in the metabolism of the cells, which is quite likely to be morphologically expressed by quantitative and qualitative changes in their cytological components.

IV. GENERAL OBSERVATIONS

1. *Acute Morphinism*

Ma,¹ in his dissertation on morphinism, divides the acute reaction to the poison into the stimulated and depressed stages. The former is 1 to 5 hours and the latter 6 to 11 hours after the rodent has been given a single injection. He is able to associate these two periods of the acute condition with accompanying constant cytological phenomena, which are characteristic for each phase. During the first period he reports an increase in the Golgi material, while the mitochondria remain unaltered. In the second, the reverse condition occurs, the Golgi material becoming decreased while the mitochondria respond by a slight increase in numbers.

Detailed examinations of the tissues under acute morphinism, in the present investigation, reveal that, although the cell inclusions in most cases respond morphologically to an acute injection, the results are so variable and inconstant, even in all good cytological

preparations, that it is impossible to associate any alterations with any special phase of the acute condition. In some tissues the cytological demarcation is so slight that it is almost impossible to distinguish between normal and morphinized material. The response, in terms of morphological variations in the cell components during acute morphinism, will now be reviewed.

Liver: An examination of Figures 1-4 demonstrates the general marked differences between the tissue fixed by drying while freezing with the new Altmann technique (Figs. 1 and 2) and the portion of the same liver treated by the ordinary histological procedure (Figs. 3 and 4). The dehydrated tissue shows less affinity for stains and, viewed through the higher powers of the microscope, resembles frozen sections of living tissue. The individual hepatic cells exhibit less shrinkage, and their nuclei, showing less selection for the stain, are larger in proportion to the size of the cell than those seen after histological treatment. The protoplasm presents a granular appearance and the mitochondria, which are preserved, can be faintly distinguished. The endothelium lining the sinusoids of blood vessels is also clearly depicted; the blood cells that stain faintly resemble those observed in the living state, the erythrocytes showing little or no shrinkage.

No gross histological differences could be observed between the acute phase (Fig. 2), 5 hours after injection, and the controls (Fig. 1), beyond a slight hypertrophy of the hepatic cells. The mitochondria and other intracellular components revealed by this technique are too poorly differentiated to observe quantitative or qualitative differences.

Figure 3 depicts a section of liver fixed in Flemming's solution and stained with iron hematoxylin 5 hours after the administration of an acute dose of morphine. A general hypertrophy is likewise observed and is apparent when compared with the normal control (Fig. 4). The hepatic cells following an acute injection invariably show an abnormal number of fat globules (Fig. 3).

Liver fixed from 2 to 10 hours after the last injection, and treated by the general cytological methods, shows a variation from the normal in the number and structure of the mitochondria in the hepatic cells. These changes accompanying the acute condition are found, however, to vary considerably, even in good cytological preparations made from different rats killed at the same time fol-

lowing morphine administration. In certain animals the response to the drug, which involves granulation and hypertrophy of the mitochondria, was so slight that it was difficult to associate any specific alteration with the acute phase. This condition was not observed in all control material, but it is assumed to be a morphological reaction expressing the sensitivity of mitochondria to the poison (Fig. 5).

The Golgi bodies, even in preparations of control liver, exhibit a wide range of structural variation. As the metabolism of this organ is complex, normal and experimental material was obtained at regular intervals after feeding. Even with these precautions it is difficult to establish a definite normal structure in osmic and silver preparations. No fragmentation of the Golgi apparatus is encountered in the morphinized tissue, but a slight hypertrophy, which is inconstant and definitely not specific for any time period following the dose, is occasionally observed.

Examination of nuclear phenomena in controls and acutely affected tissue reveals no deviation from the normal type.

Pancreas: Pancreatic tissue, when treated by the new Altmann freezing method, responds well to this technique. Far less shrinkage is observed, as compared with the ordinary histological controls. The filamentous mitochondria and zymogen granules are detected but are not clearly differentiated from the cytoplasm, thus making quantitative or qualitative estimations impossible. The islets of Langerhans display less affinity for the stain than the acinar tissue. No gross histological differences can be ascertained between normal material and that under acute morphinism.

Control cytological tissue, after fixation in Regaud's fluid and staining with acid fuchsin and methyl green, is represented in Figure 7, where the deeply staining zymogen granules are seen aggregated within the nuclear zone, while the filamentous mitochondria are restricted to the basal regions of the acinar cells. Figure 6 depicts the experimental material, $5\frac{1}{2}$ hours after injection, treated by the above method, and shows clearly an extreme variation in the action of the drug. In most areas the filamentous mitochondria have rounded up into granules which occasionally fuse, forming larger globules. These globules stain poorly and resemble similar structures obtained in the phosphorous poisoning experiments of Scott²⁴ from the mitochondria of the acinar cells of the guinea pig.

This phenomenon is more marked in some preparations than in others. Examination of a large number of sections demonstrates enormous variations, from a slight abnormality to extreme globule formation. The mitochondria in the islet cells appear to show more resistance to changes. Although the mitochondria in the pancreas of different rats under acute morphinism do not give a constant cytological picture, their general reaction appears to be more specific than in liver cells under similar conditions.

The Golgi apparatus does not show very significant changes when compared with the control material. Slight fragmentation is occasionally met with in the experimental tissue, but no marked increase in the amount of Golgi material, described by Ma during the "stimulated phase," is encountered. Similar variations are observed in tissues 2 hours after an acute administration, at which time the reaction is similar to that produced 5½ hours after the dose. The gross cytological metamorphosis pictured by Ma between these two time periods is not confirmed.

Nervous Tissues: The resistance of mitochondria to pathological conditions in nerve cells, which has been previously commented upon, has been confirmed in this investigation. The cells of the spinal ganglia, anterior horn cells of the spinal cord and the large pyramidal neurones in the cerebral cortex, together with the Purkinje cells of the cerebellum, were examined at various intervals following an acute dose. The mitochondria of these cells of the nervous system display no structural or topographical deviation from the normal. Even in material selected from a rodent that had received a second injection, 3 hours after the first failed, there are no significant alterations. Under these conditions no changes in either the arrangement or staining reactions of the Nissl substance were recorded.

The Golgi apparatus, in contrast with the mitochondria, reveals a variable response to the action of the morphine in the form of a slight hypertrophy and occasional fragmentation. However, the apparatus shows great morphological variation in most nerve cells, even under normal conditions, and varies from rod-shaped bodies to a network formation. This makes it difficult to distinguish any reaction of the neurone to changes in environment of the Golgi apparatus in terms of structural alterations. The hypertrophy and fragmentation is at times most marked (see Figs. 19, 20 and 21) when compared with control material (see Figs. 16, 17 and 18).

These results partly confirm those of Ma, who detected a similar increase in amount during the "stimulated phase" of acute morphinism, but they are contrary to the recent findings of MacEwen and Buchanan,³⁰ who failed to correlate any deviation from normal in nerve cells under similar conditions. Figures 16 to 18 depict the structure of the Golgi bodies in normal healthy neurones which, in all well controlled preparations, present a broken network of dense anastomosing threads. The apparatus in all larger cells displays a tendency for the network to become dispersed throughout the cytoplasm (see Figs. 17 and 18), while in the smaller cells it appears to be more closely drawn together in a compact mass around the nucleus (Fig. 16). Spinal ganglia, fixed 1 to 5 hours after morphine injection, show that acute poisoning is associated with a slight increase or hypertrophy of the Golgi material. This phenomenon, which is sometimes accompanied by fragmentation of the Golgi bodies, is depicted in Figures 19, 20 and 21. Further observations upon the spinal ganglia during Ma's "depressed period" (10 hours after injection) fail to confirm the contentions of this author. No decrease in the Golgi material is detected in tissues fixed before or after the "depressed period."

Careful studies of a great number of preparations reveal conclusively that acute poisoning is associated with a slight hypertrophy and occasional fragmentation of the Golgi apparatus, the physiological interpretation of which will be a subject of discussion in a later portion of this communication.

Intestine and Stomach: The mitochondria, under normal conditions and during acute morphine poisoning, were extensively studied in the absorbing columnar epithelium of the villi in the small intestine, which lends itself beautifully to cytological investigation. These inclusions within the normal epithelial cells are arranged longitudinally to the axis of the cell, presenting a wavy filamentous appearance (Fig. 8). In the basal or subnuclear region of the cell the mitochondria tend to aggregate and form a capsule-like structure around the nucleus, which is oval in shape and situated in the middle portion of the cell. The accumulation within the subnuclear zone, which consists of granular mitochondria, is apparently a surface tension effect due to their phosphatidal nature.³¹ Material fixed after an acute injection reveals small changes in shape and size of the mitochondria. These alterations vary greatly from rod-

shaped structures to globules in preparations taken from different rats at the same time following an acute dose. The most constant feature, in the acute condition, appears to be hypertrophy of the mitochondria. An extreme breaking down and rounding off of these structures is shown in Figures 10 and 11, and contrasts with the normal condition seen in Figure 8. Extensive studies have demonstrated that acute poisoning cannot be definitely associated with any special changes in the form of the mitochondria beyond a tendency for the filaments to break down into polymorphic bodies. It must be pointed out that the mitochondrial structures vary according to the normal functional activities of the cells, and great care must be exercised not to confuse this issue with alterations in shape and size incurred as the result of the drug. It has been shown, for instance, that mitochondria in the epithelial cells of the villi in man alter from the filamentous condition into rod-shaped bodies during fasting. Therefore, it is essential to regulate the diet of the animal as well as the time of collecting the material, before ascertaining any reaction to induced pathological conditions.

The response of the Golgi apparatus to the drug can be observed best in the gastric glands of the stomach, as well as in the glands of Brunner in the submucosa of the small intestine, and can be studied to advantage when sections are cut with a slight obliquity to the long axis of the gland. Under normal conditions the individual cells forming these glands are large, their nuclei being situated in the basal region, while the Golgi apparatus is always seen in the clear area of cytoplasm between the nucleus and the apical region. The mitochondria, when in the filamentous condition, take up a position at the opposite pole of the cell and invariably form a dense mass around the distal region of the nucleus. At the onset of secretory activity the Golgi bodies, which are present in the form of individual polymorphic structures, become reduced in amount and the mitochondrial filaments give rise to granules which migrate with the secretion droplets toward the apical regions of the cell (Fig. 14). This phenomenon has led many workers to believe that the Golgi and mitochondrial substances, by means of chemical interaction, play a dominant rôle in secretory activities, and also stresses the care that should be taken to determine their normal functional variations, before relying on morphological changes as indicators of physiological depression.

Detailed examinations of the cardiac glands of the stomach, pylorus and those of Brunner in the submucosa of the duodenum, obtained at the same time after feeding and at various intervals following an acute injection, reveal that the action of the drug incurs a slight hypertrophy of the Golgi bodies (Fig. 12). This phenomenon is extensively variable and is more marked in some areas of the same preparation than in others, but is not due to imperfect impregnation. In several instances this process is followed by fragmentation of the Golgi material, as illustrated in Figure 12. Extensive studies of the Golgi apparatus under acute conditions of morphinism demonstrate its inconsistent morphological reaction to the poison.

Muscle Tissue: Preparations of skeletal, cardiac and the involuntary muscle in the walls of the intestine show no cytological deviation from the normal type. The apparent fluctuations in the amounts of Golgi and mitochondrial substance, described by Ma, are not encountered.

Thyroid: The cell components of this gland display no cytological response to acute poisoning, beyond an increase in the amount of fat in the vesicular epithelium following a second injection 8 hours after the first. The secretory polarity of the Golgi apparatus, under these conditions, is maintained.

Kidney: A tendency for the short mitochondrial filaments in the epithelium of the convoluted tubules to break down into globules is a phenomenon generally associated with the acute condition. This reaction is more variable even in tissues obtained from the same rodent, and on the whole is most marked 8 hours after injection. The larger globules stain a bright pink with the acid fuchsin-methyl green technique, and various intermediate colors between the red-staining mitochondria and these structures can be seen. Similar staining gradations are detected with the other methods. The Golgi bodies, as far as could be ascertained, are unaltered. But as structural variations under normal functional conditions cover a wide range, the difficulty in associating specific alterations in the form of the apparatus in this organ with induced poisoning is apparent.

The cytological changes produced by the action of morphine, described above, were not found to be similar in different tissues, as Ma contends, nor could any abnormal nuclear phenomenon be attributed to the morphine.

The behavior of the rats to acute doses of morphine hydrochloride appears to be a controversial point. Ma holds that they show symptoms of slight irritability, while MacEwen and Buchanan³⁰ report that their rodents, after a similar dose, pass into a deep narcosis for several hours, after 3 to 5 minutes following the injection. My observations, however, entirely confirm those of Ma.

2. *Chronic Morphinism. Cytopathology of Chronically Morphinized Tissues Examined at Intervals Before Discontinuation of the Drug*

When discussing the pathology of morphine tolerance Ma contends that, provided the daily dose is administered regularly, no cytological deviation from the normal occurs, although he describes certain fluctuations in the form of specific rhythmic changes in both amount and form of the cell components during the interval between injections. During what Ma terms the "middle of the craving period satisfied," which is 12 hours after the last injection, a general dispersal and increase in the Golgi and mitochondrial material is reported. It is further contended that microscopic preparations obtained from rats at the end of 24 hours, before they receive their next regular dose of morphine, contrast cytologically with those of 12 hours earlier. In the interim a decrease in the amount of Golgi material occurs and the inclusions gradually return to their normal morphological state.

The evidence derived from this investigation does not confirm that of Ma. Certain slight changes in the morphology of the cell organs, which were by no means specific to all tissues, nor to any period either before or after the regular injection, were observed.

Examination of tissues taken from rats that were under chronic morphinism for 6½ to 7 months revealed the following cytopathological changes.

Stomach and Duodenum: Figure 13 represents a transverse section through a gland of Brunner in the submucosa of the duodenum. In the vast amount of material studied the Golgi bodies in these gland cells possess a depleted appearance and contrast with similar tissues under normal and acute conditions (compare Fig. 13 with 12). This picture reveals an extreme variation of the apparatus in the chronic state, and also shows the loss of power for selective staining

as well as morphological change. Both in the normal and acute phases it tends to exist as a compact polymorphic body situated within the apical zone of the cell. In the chronically morphinized tissue, however, the depleted Golgi substance varies from small irregular bodies to anastomosing threads, which do not confine themselves entirely to the apical regions, as in the control preparations, for they are frequently detected lying around the basal aspect of the nucleus (Fig. 13).

The reaction of the mitochondria to chronic poisoning is demonstrated clearly in the absorbing columnar epithelium of the villi. These structures are usually filamentous and orientated longitudinally to the axis of the cell in normal material (Fig. 8). In chronically morphinized tissues (see Fig. 9) they are extremely variable, tending to become fragmented, forming irregularly shaped bodies, somewhat depleted in appearance and finally losing their polarity in the cell. It appears likely that these phenomena, especially the loss of polarity, are due to chemical alterations in the structure of the mitochondria.

Cowdry³² has admirably summarized the evidence for concluding that the cytoplasmic organs are sensitive to pathological conditions, and the cytological deviations from normal occurring in these gland cells are further confirmation of this sensitivity. Slight departures from the normal up to the extreme condition pictured in Figure 9 are common in this chronic material, taken at any period between the regular injections. This again demonstrates forcibly the enormous limits of structural variation which make it impossible to link any specific change with any special degree of chronic poisoning.

Nervous Tissues: Owing to the complex and diverse morphology of the Golgi apparatus, even under normal conditions, it is only with difficulty and caution that any cytological changes can be associated with any degree of morphine tolerance in nerve cells. Beyond an aptness for the apparatus, in well controlled preparations, to display less affinity for the several stains, no abnormality was met with.

The curious resistance of mitochondria to pathological conditions in neurones is again confirmed by a study of their behavior in the chronically morphinized material.

Pancreas: In many preparations of this tissue small departures from the normal in the cell components, which were confined to the

experimental material, are encountered. The mitochondria retain their normal filamentous appearance. This lack of response is not found in the case of acute poisoning (Fig. 6). At the same time they show less absorption for the stains, a phenomenon that appears to be characteristic of the chronic state. Slight fragmentation of the apparatus is occasionally met with, but the marked increase and dispersal of this substance within the apical portion of the acinar cells, in the form of small granules, reported by Ma, 12 hours following the last morphine administration, is not apparent.

Thyroid: Certain interesting pathological changes in this gland appear to be associated with the chronic poisoning. The vesicles, in the majority of cases, contained visibly smaller amounts of colloid, a phenomenon that becomes significant when compared with glands from control rodents. The cytological constituents retain their normal morphology and staining reactions. The accumulation of fat characteristic of the acute condition is not detected, and the lipoid droplets generally seen in the epithelium of the healthy thyroid are entirely absent. The interstitial connective tissue shows no significant variations.

Kidney: The mitochondria present in the renal epithelium of the convoluted tubules are well developed and resemble those seen in the normal organ, except that in certain areas of most preparations the same inability to absorb the stains is met with. Globules, similar to those detected under acute morphinism, are present in a modified degree. The Golgi bodies display an irregular behavior by fragmenting in certain localized areas in the epithelial cells, and come to resemble the structures pictured by Ma during the middle of the "craving period satisfied" of chronic morphinism.

Liver: Preparations of the liver show the mitochondria present in a depleted form, otherwise their morphology and distribution within the hepatic cells appear normal. Variable decreases in the glycogen content of these cells is a conspicuous feature in many regions of chronically morphinized liver.

In view of these results it is of interest to review the observations of several authors in this field. Arnold²² was one of the earlier workers who found an association of glycogen formation with mitochondrial activity. This work is not in accord with the later contentions of Bang and Sjövall,²³ who found that the distribution of glycogen within the cell protoplasm does not conform to the

distribution of the mitochondria. The previous observations of these authors were later confirmed by Mann³⁵ in 1928, who likewise discovered no evidence of a morphological relation between the mitochondria and the glycogen of liver cells. Kater³⁶ in a recent paper has come to the conclusion that there is no evidence for associating the deposition of glycogen in the liver cell with mitochondrial activity. In view of this conflicting evidence it is impossible to determine whether the depletion of the mitochondria, characteristic of liver under chronic morphinism, can be correlated with the decreases in glycogen content. The Golgi material failed to show any reaction to chronic poisoning.

Muscle Tissues: Skeletal and cardiac muscles reveal no significant cytopathological changes that can be attributed to the effect of continuous morphine administration.

3. *Behavior of the Golgi and Mitochondrial Material in Addicts, After Abrupt Withdrawal of the Drug*

The "craving period" of chronic poisoning after withdrawal of the drug is divided by Ma into two phases, the first 1 to 4 days and the second 4 to 6 days after the final injection, and he finds that each period is definitely indicated by some cytological change. The first phase of the "craving period" is marked by such a decrease in the amount of Golgi substance that, in some cells, he reports it as almost absent. The second period is marked by a reappearance of the Golgi material and an increase in the mitochondria, which are enlarged and display better affinity for the stains. At the end of 10 to 12 days these inclusions return to their normal state.

Detailed microscopic examination of the form and fluctuation of the cytological constituents in the tissues of the addicts during these phases reveals that the morphological variations encountered are not necessarily typical of any special state. They are found to exist in different areas of preparations from the same piece of chronically morphinized tissue, and therefore come within the limits of possible variations of the Golgi apparatus and mitochondria in this material. Similar limits of variation may be found occasionally in the cell components in any tissue, even in the normal state.

Observations were made upon the tissues of addicted rats at different intervals from 24 hours until 3 weeks after sudden discon-

tinuation of morphine. Although it is known that abrupt withdrawal leads to severe pathological conditions such as edema, in many tissues, this investigation has shown, contrary to Ma, that it is impossible to correlate any specific cytological state with these phenomena, and no marked cytological demarcation in the form of a sudden decrease of the Golgi lipid (as Ma terms it), even in preparations 48 hours following discontinuation, was detected. Edema incurred by morphine withdrawal, according to Barbour, Hunter and Richey,³⁷ is accompanied by rapid loss of lipid in most tissues. This should correlate theoretically with Ma's findings, when taking into consideration his chemical interpretation of the lipoidal structure of the Golgi apparatus.

Even in tissues such as the gastric glands of the rat of 6½ to 7 months addiction, in which the cell components appear to react to the drug in terms of a variable depletion of the Golgi material, no conspicuous cytological differences can be detected at any interval immediately following withdrawal. The same is found to be the case in tissues that give no significant response to continuous administration of the poison.

Extensive studies have shown that the limits of morphological variation of the cell inclusions seen in the same piece of chronically morphinized tissue are almost identical with those Ma obtained in any particular phase, either during or after the period when the regular morphine injections were withheld.

4. *Microincineration of Normal and Experimental Tissues*

The location of the mineral constituents in normal, acute, and chronically morphinized tissues both before and after withdrawal, as revealed by the method of microincineration of Policard,³⁵ was extensively investigated. After burning away the protein compounds the distribution and orientation of the inorganic salts were studied in dark-field illumination.

Although thorough examination of the incinerated material shows a specific distribution of the inorganic residues, which are visibly constant in cells forming similar tissues, no marked departures from the normal are observed between the experimental and control material.

Certain abnormal ash distributions are, however, occasionally met with, but repeated experiments demonstrate that this phenom-

non is due, in all probability, to an artificial clumping of the mineral constituents brought about during the process of burning—a factor that appears to be largely dependent upon the temperature and time of incineration.

Nevertheless, definite changes in the amounts and localization of mineral salts in various pathological states have been reported.^{39, 40, 41} The most striking results, in view of the negative ones obtained in this investigation, are those of Policard, Noël and Pillet.⁴² These authors comment upon the effect of different diets on the mineral distribution in incinerated liver sections from white mice. Variations from the normal were found after feeding the rodents on strict sugar, protein and fat diets, and also in the rate at which these tissues incinerated.

This significant mineral variation, resulting from the changes in diet, observed by these authors, is interesting as no inorganic disturbance, following a lecithin diet of rodents for long periods, was detected by incineration. In the case of lecithin feeding this might be expected, for Glikin,⁴³ Burow,⁴⁴ Altschul,⁴⁵ and Stern and Thierfelder⁴⁶ concur that iron and calcium are present in lecithin in appreciable amounts.

The administration of lecithin is followed by an increase in the lecithin content of the liver,⁴⁷ but incinerated sections of this organ from rats fed for over 4 months on a lecithin diet fail to reveal any differences in mineral localization when compared with liver obtained from control rodents fed on a regular diet.

Appreciable variations in the amounts of inorganic salts in normal and corresponding pathological tissues estimated by chemical analysis have likewise been described. The most noteworthy researches in this sphere are those of Eaves,⁴⁸ who gives interesting data concerning the increase in mineral salts during many pathological conditions in the human brain, and that of Adams,⁴⁹ who discovered that serum calcium is higher in the blood of rabbits suffering from senile cataract than in normal animals of the same age.

As it has been shown that well advanced morphine addiction not only causes dehydration in certain internal organs, but that sudden withdrawal of the drug results in a remarkable redistribution of water and severe pathological conditions such as edema of the brain, liver, kidney and muscles, as well as a marked increase in blood calcium, one would naturally expect such changes to be indicated by

a disturbance of the mineral organization of the cells. Microincinerated preparations, however, of liver, kidney and brain, which were pathologically affected by sudden morphine withdrawal, show no deviation from the normal controls. Even if the pathological changes incurred by morphine poisoning are inorganically expressed by small specific fluctuations of the mineral constituents, it is unlikely that any such slight variations could be detected by the present microincineration technique, although a recent paper by Scott⁵⁰ on quantitative estimations of the ash in incinerated preparations indicates possibilities in this direction.

5. *The Cytological Effect of Lecithin Feeding on Normal Tissues, and on Acute and Chronically Morphinized Tissues, both Before and After Withdrawal of Morphine*

The biological significance of lecithin feeding in normal animals, as well as its effect upon the toxicity of morphine and other alkaloids, has been a subject of much controversy. That lecithin administration results in an increase in the general body weight and acts as an agent stimulating normal growth is a matter of debate.

Ma, when investigating the physiological results of lecithin feeding on the influence of body weight in white male rodents, reported a significant increase. After one month his experimental animals showed in some instances an increase of 9 gm. over the controls. A still greater increase of 27 gm. was found in the lecithin-fed females, as compared with similar females of the same age maintained on the control diet.

Ma attempts to correlate this increase in weight, following yolk-lecithin feeding, with definite cytological phenomena. His preparations showed that the cell components are better developed in tissues from normal lecithin-fed rats, the most marked differences being exhibited by the Golgi material. Ma's physiological interpretation of this phenomenon is based on the supposition that the Golgi substance consists mainly of lipoids, among which lecithin occupies the first place. The fact that lecithin is not absorbed as such will be discussed later.

The growth experiments with lecithin feeding, in this investigation, yielded negative results. Twelve rats (6 males and 6 females) of the same age and weight were segregated and fed for 4 months on

Merck's yolk-lecithin, in proportions as indicated in the text. At the end of this period their weights were compared with corresponding controls fed on the regular diet, and no significant increases in either their growth or weight were recorded. It might be suggested that the vast differences obtained by Ma between his lecithin and control-fed rodents are explicable on the grounds that the control diet was inadequate. Slight variations in body weight, however, were detected between the chronically morphinized rats of 6½ months addiction and those fed upon an ordinary diet. The loss of weight in these addicts was more marked after the first 2 months and on an average never exceeded 4 to 5 gm. Whether this was directly due to the pathological action of the morphine, or secondary effects, associated with a significant loss of appetite, is difficult to ascertain. After 4 months of morphine administration these rodents developed a normal appreciation of food, which was followed by a rapid increase in body weight. It is interesting to record that Barbour, Hunter and Richey,³⁷ when observing the reaction of dogs to morphine addiction, found that the animals always refused their food during the commencement of a series of injections.

Microscopic examinations of control tissues again demonstrate the enormous limits of cytological variation, particularly expressed by varying amounts of the Golgi substance in normal material. These variations make it exceedingly difficult to determine any relative increase of the cell components in the experimental tissues that might be incurred as the result of lecithin feeding.

Another important factor to be taken into consideration when making quantitative estimation is the metabolic state of the cell at the time of examination. Figure 14 depicts a transverse section through the gastric gland of a lecithin-fed rat killed at the onset of secretory activity, in which an increase in mitochondria and associated granules, together with a decrease in Golgi substance, is involved. Observations on such material during the resting stage of glandular activity and also in other tissues show nothing significantly above normal. The cytological constituents in lecithin-fed tissues are always clearly rendered, and in several instances better developed than in the controls, but as this occasional phenomenon is an irregular one it cannot be attributed solely to the lecithin diet.

In order to restore the Golgi apparatus, which Ma found "almost entirely lacking" during the "craving period," following morphine

withdrawal, he contends that if lecithin is regularly administered together with the usual dose of the drug a few days before discontinuation, the Golgi material is thus prevented from becoming reduced and so retains its normal appearance. He shows cytologically that without lecithin treatment the cell inclusions revert to their normal condition 10 to 12 days after the final injection, but that lecithin feeding, on the other hand, 6 days before and after, prevents this abnormal reduction of the Golgi substance.

As material studied in this investigation reveals that the apparatus, and in some instances the mitochondria, in chronically morphinized tissues undergo a depletion before withdrawal (see Fig. 13), it is difficult to compare the results of subsequent treatment with those obtained by Ma. Experiments, nevertheless, were carried out in this direction, but no significant results were observed. Chronically morphinized tissues from rodents, one-half of which were control-fed and the other lecithin-fed, 8 weeks before morphine discontinuation, were examined 2 days after sudden withdrawal, and no cytological differences beyond the normal morphological variations typical of any healthy cells were observed.

Figure 15 represents the form of the Golgi apparatus, typical of the gastric glands, of a 6½ months addict, lecithin-fed and killed 4 days after withdrawal of morphine. The apparatus has in no way responded to lecithin treatment. It is still reduced in amount, having a slightly depleted appearance, and in no way differs from that of the control-fed material obtained under similar conditions. Extensive examination of various tissues yields no significant results.

The cell inclusions that reacted to the chronic poisoning in the several organs gradually return to their normal state within 2½ weeks after the final injection, which confirms the previous observations of Ma.

The immediate effect of an acute morphine injection upon the Golgi material, of nerve and glandular tissues of a normal lecithin-fed rodent, shows that beyond a slight hypertrophy of the apparatus, frequently observed under such conditions, no cytological deviation from type is detected which would suggest any interaction between the alkaloid and the lecithin.

V. DISCUSSION

A review of the behavior of the several cell organs during acute and chronic poisoning, as well as their reactions to cell injury and disease, shows that they are responsive to gross metabolic and pathological changes, but their use as indicators of fine degrees of physiological depression is not based on sufficiently reliable evidence. When taking into consideration the wide range in the limits of morphological variation of the inclusions, found even in normal healthy cells, it is impossible to correlate any slight structural alteration with any specific degree of morphine tolerance, and on these grounds this investigation fails to concur with the results obtained by Ma. The Golgi elements, in particular, have been shown to be inconstant morphological units, their structure depending largely upon their reaction to the type of technique employed. The apparatus, as seen even in well controlled preparations, in the same piece of tissue treated by either the osmium and silver methods, invariably shows structural diversity.

These experiments also demonstrate that the appearance of the Golgi elements is not only contingent upon the impregnation, but also the period of fixation which had to be regulated according to the type of tissue. Some of the variable factors controlling the impregnation of the Golgi material have recently been discussed in detail by Nahm.²⁹

The mitochondria appear to be more consistent in their reactions to different cytological procedures and to induced abnormal conditions. Their extraordinary resistance to acute and chronic poisoning in nerve cells is difficult to interpret, especially when variable morphological changes are associated with this phenomenon in other tissues. These results confirm those of Strongman⁵¹ and McCann,⁵² the latter finding that mitochondria in experimental poliomyelitis remained unchanged even after the nerve cells had undergone partial degeneration, while the former demonstrated their immutability in functional exhaustion.

The hypertrophy of the cell organs following administration of an acute dose of morphine, which is very noticeable in the Golgi elements of the nerve cells of the spinal ganglia and in the mitochondria in the acinar cells of the pancreas, is a difficult phenomenon to explain in view of the uncertain chemical composition of these

inclusions. Bancroft and Richter,⁵³ when inquiring into the physiological action of anesthesia on tissues, suggested that narcotics such as morphine caused an aggregation of the cell colloids. This is worthy of consideration and the possibility that an absorption of the alkaloid might lead to an increase in the surface area of these cell constituents should not be overlooked.

The depletion of the Golgi and mitochondrial material, expressed variably in terms of partial loss of staining reaction combined with a slight decrease in their bulk, typical of most chronically morphinized tissues, is an interesting gross cytological response that might possibly be correlated, in some respects, with the physiological observations of Barbour, Russell, Flowers, Dunham and Hunter.⁵⁴ These investigators find that well advanced morphine addiction incurs dehydration of certain of the internal organs, and also that sudden withdrawal of the morphine causes edema, with loss of lipoids, in some parts of the brain, liver and kidney, together with a remarkable redistribution of water.

Assuming that the generally accepted idea of the lipoid structure of the Golgi substance is correct, this might account for the depletion of the apparatus after morphine discontinuation, but as it seems to take place in certain tissues of well advanced addicts, before the drug is withheld, it is difficult to interpret.

Barbour, Hunter and Richey⁵⁷ report an interesting observation in this connection. They discovered that when fat-fed dogs were suddenly deprived of morphine a rapid loss of liver fat (in 4 days diminished by 36 per cent in some cases) occurred, while the livers of dogs on a non-fat diet showed an increase. This observation is of interest in view of the recent work of Smith¹⁵ and Kater and Smith,¹³ who show that mitochondria in liver cells probably act as catalysts, stimulating a synthesis of fat from the fatty constituents of the cell. It is therefore possible that the reserve fats of adipose tissues in the dogs fed on a non-fat diet are conveyed by the blood stream to the liver cells and here resynthesized by mitochondrial activity.

Overton⁵⁵ finds that the power to produce narcosis is entirely dependent upon the power of the substance to dissolve the lipid membrane of the given cell. As it is seen that the cytopathological response to morphine varies according to the tissue acted upon, it might be suggested that this difference in effect is produced by the

fact that the chemical structure of the cell membrane varies with certain types of tissues and gives a different response to morphine in each case.

The results presented in this paper on the negative effect of lecithin administration in normal, and in chronically morphinized rodents, both before and after morphine withdrawal, become more explicable after reviewing the evidence of several workers in this field.

Lecithin, which belongs to the group of phospholipoids, was first isolated by Gabey in 1846, and on hydrolysis yields glycerophosphoric acid, fatty acids and choline.⁵⁶ Following its discovery it was used clinically, and later abandoned, as a cure for diseases ranging from beriberi and scurvy⁴⁷ to anemia and debility,⁵⁷ and its influence as a growth-stimulating factor is still a matter of controversy. Ma,⁵⁸ however, reports success in applying this substance to suppress symptoms during treatment of human opium addicts, and also finds that on microscopic examination of their blood the Golgi apparatus in leukocytes, during the "craving period," decreases considerably in amount, and increases provided the patient is treated with lecithin. In a later investigation⁵⁹ he finds that the erythrocytes of chronically morphinized rats "received great benefit from the treatment with lecithin after withdrawal."

This last work of Ma is of interest in view of the recent observations of Masasue,⁶⁰ and Grönberg and Lundberg.⁶¹ Masasue finds that blood taken from human morphine addicts, when compared with that of normal persons, shows a lower red count. Grönberg and Lundberg have found independently that lecithin administration *in vivo* brings about a rise in the number of red blood cells. From this evidence it might be assumed that morphine has a depressing effect while lecithin stimulates erythropoiesis in bone marrow. It has been shown further that, in the case of advanced human morphine addicts, a single injection of the drug results in a leukocytosis within 30 to 60 minutes. As this change of leukocytosis, however, is only a transient phenomenon (the blood becomes normal in 90 to 120 minutes following the injection), it is difficult to correlate this physiological change with the cytological changes described by Ma,⁵⁹ as occurring in the blood of chronically morphinized rats for extended periods after withdrawal.

MacLean,⁴⁷ in his monograph, "Lecithin and Allied Substances," admits that the results of feeding experiments with lecithin are in-

conclusive, and that its administration does not appear to be followed by very significant results. Other authors point out that owing to the unsatisfactory state of knowledge concerning the lipins it follows that their exact function in animal economy must necessarily be obscure.

Ever since Danilewsky in 1895 published his startling results in which he found that tadpoles placed in water containing 0.07 per cent lecithin gained 300 per cent more weight than controls kept in ordinary water,⁶² many workers have come forward with most conflicting evidence concerning the growth-stimulating powers of this substance. Ma's results entirely confirm those of Hatai,⁶³ who discovered that white rats, which received lecithin either by injection or mouth, gained in body weight more rapidly than the control-fed animals; the gain in the experimental rodents being on an average 60 per cent. Likewise, Desgrey and Zakey⁶⁴ describe an increase in the body weight, especially in the nervous and skeletal systems, following injections of lecithin. It is interesting to note that these conclusions were not entirely confirmed by Hatai, who further found that administration by mouth incurred greater gain in body weight than by inoculations. Then Goldfarb⁶⁵ reported that there was no clear evidence of growth stimulation as a result of administration of this substance. He entirely failed to confirm Danilewsky's work on tadpoles, and after extending these experiments to both carnivorous and herbivorous mammals failed to concur with the previous results of Hatai, Desgrey and Zakey.

A more recent publication on this subject by Brachiesi⁶⁶ gives an independent view on the nature of lecithin stimulation of growth, which differs from the findings of Ma and other authors. Brachiesi treated guinea pigs with subcutaneous injections of lecithin for 15 successive days. The animals were killed after 18 days. The lecithin-fed animals showed a decided increase in weight over the controls up to the 12th day. Their weight then became constant for the next 6 days. From these experiments this author assumed that lecithin modified the metabolism up to the 12th day and that afterwards an equilibrium was reached. Other groups of animals were similarly treated over longer periods and the same phenomenon was encountered.

There is, in fact, evidence that suggests the nature of the influence of lecithin on growth is rather inhibitory. Robertson⁶⁷ found that

large doses of lecithin given to mice produced a slight retardation of growth. Similar retardation in suckling mice, following the administration of 100 mg. of egg-lecithin per day, given by mouth to the mother, was recorded by Robertson and Cutler.⁶⁸

This result is supported by the evidence of Fingerling⁶⁹ who contended that the addition of lecithin to the diet is without effect on the secretion of milk.

The specific nature of the fatty acids of lecithin was first doubted by McCollum, Halpin and Drescher,⁷⁰ who demonstrated that the degree of unsaturation of the lipin fatty acids of egg yolk is accidental and can be easily influenced by diet. If this is so it might help to explain many of the conflicting results on the growth phenomena in relation to this substance obtained from eggs.

In view of the results of these workers and those obtained during this investigation the following experiments of MacArthur and Luckett⁷¹ are of interest. These investigators succeeded in eliminating not only lecithin but cephalin, cholesterol and fat from the indispensable constituents of foods for mice, and Osborne and Mendel⁷² found that the body weights in rats could be maintained on a fat-free diet.

The recent cytological findings of Ikeda⁷³ on the effect of lecithin on the Golgi and mitochondrial elements in chicken material do not agree with those of Ma, and the present investigation. This author reports that lecithin inhibits the development of the Golgi apparatus, but has the opposite influence on the mitochondria. The results of Okada,⁷⁴ on the effects of lecithin upon the mitochondria in the neurones of rabbits, confirm those of Ikeda.

When reviewing the several conflicting observations of these investigators the fact that lecithin is not absorbed as such raises a question which Ma admits is difficult to answer. MacLean⁴⁷ showed that it is acted upon by lipase within the intestine and broken down into fatty acids, glyceryl-phosphoric acid and choline. This may also have a bearing in some degree on the negative results obtained by lecithin administration, both as a growth-stimulating substance and a therapeutic agent for the treatment of morphine addiction in rats.

The histological changes in the thyroid gland under acute and chronic morphinism, recorded in this study, do not coincide with those of Scarborough.⁷⁵ This worker finds that chronic poisoning

is not associated with any change in the structure of this gland and, moreover, that the combination of chronic morphine poisoning with thyroid feeding inhibits the action of the drug upon the nervous system and checks the edema of the internal organs, generally associated with this condition.

When correlating cytological changes with pathological depression the fact that the cell inclusions are morphologically conditioned according to the chemical state of the cell must first be considered. As has been shown, the reduction of the Golgi material, enspherulation and increase of the mitochondria, are all part of a normal phenomenon associated with the general functional behavior of the gland cells during secretory activity. Even in healthy non-glandular cells, in both the animal and plant kingdoms, fluctuations in the amount of the inclusions, which are probably morphological indications of a synthesis involving condensation of material within them or at their surfaces, are frequently encountered. As physico-chemical processes within the cell appear to be largely responsible for the enormous limits of structural variation of cytoplasmic constituents, seen in normal tissues, these experiments have emphasized the danger of relying on these variations for gauging fine degrees of physiological change in cells undergoing diverse chemical influences.

VI. SUMMARY

1. An investigation has been made of the reactions of cytoplasmic structures in different cell types to varying degrees of morphine poisoning, during the periods of regular injections of the drug and at stages following its abrupt withdrawal.

2. Under conditions of acute morphine poisoning the cell inclusions show variable morphological changes, which are described in detail, but specific changes, indicative of special phases of the acute condition, could not be recognized consistently.

3. More significant, but still inconstant, alterations in the cytoplasm follow addiction for periods of 6 months or more and before the injections are discontinued. In the glands of the stomach and duodenum the Golgi apparatus is depleted and the mitochondria are frequently fragmented and without regular polarity. Minor changes are recorded in the pancreas, thyroid gland and liver.

4. Abrupt withdrawal of morphine, after a prolonged period of addiction, has been shown by other investigators to cause patho-

logical conditions in various tissues. There is no evidence, however, of cytoplasmic alteration which would indicate the effects of withdrawal of the drug.

5. Morphine poisoning does not cause alterations in the mineral constituents of tissues that can be detected by microincineration, although this technique has been successful in the investigation of various common pathological conditions.

6. The growth-stimulating action of lecithin fed to normal rodents is not confirmed, nor does this substance appear consistently to alter the cytoplasmic structures in normal tissues. Lecithin administered therapeutically to acutely and chronically morphinized animals, both before and after withdrawal of the drug, has no significant cytological effect which would suggest that there is any interaction between itself and the morphine.

7. The pathological action of morphine on cells in various tissues appears to occasion changes in the cytoplasm that are so delicate and variable that morphological alterations in cell structures, such as the Golgi apparatus and mitochondria, are not reliable indicators of the acute, chronic and withdrawal phases of morphinism.

8. Morphological changes in the cell organs are met with in special instances following morphine poisoning, but when the limits of variation of the cell organs, which alter even in different functional states of the normal healthy cell, are recognized, these changes are found to range within such limits and therefore lose significance as indicators of the different phases of morphine poisoning.

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DESCRIPTION OF PLATES

PLATE 71

- FIG. 1. Control section of liver from a normal healthy rodent after treatment by the Altmann technique for fixation by drying while freezing, stained with Heidenhain's hematoxylin and counterstained in eosin. The hepatic cells show less shrinkage when compared with those of a portion of the same liver, fixed by the ordinary histological procedure, and their nuclei show less affinity for the stain.
- FIG. 2. Showing section of liver, fixed by the Altmann method, 5 hours after a single acute injection of morphine sulphate. Observe the slight hypertrophy of the tissue.
- FIG. 3. Same material morphinized as in Fig. 2, but fixed in Flemming's solution and stained by Heidenhain's hematoxylin instead of by the Altmann method. Note the accumulation of fat in the hypertrophied hepatic cells, which is a phenomenon associated with the acute condition.
- FIG. 4. Showing section of control liver obtained from a normal healthy rodent, after treatment by the ordinary histological procedure. The differences in staining as well as the general condition of the cells are apparent when compared with similar material treated by the Altmann technique, as demonstrated by Fig. 1. Note the absence of cell hypertrophy and fat accumulation typical of acutely morphinized liver.

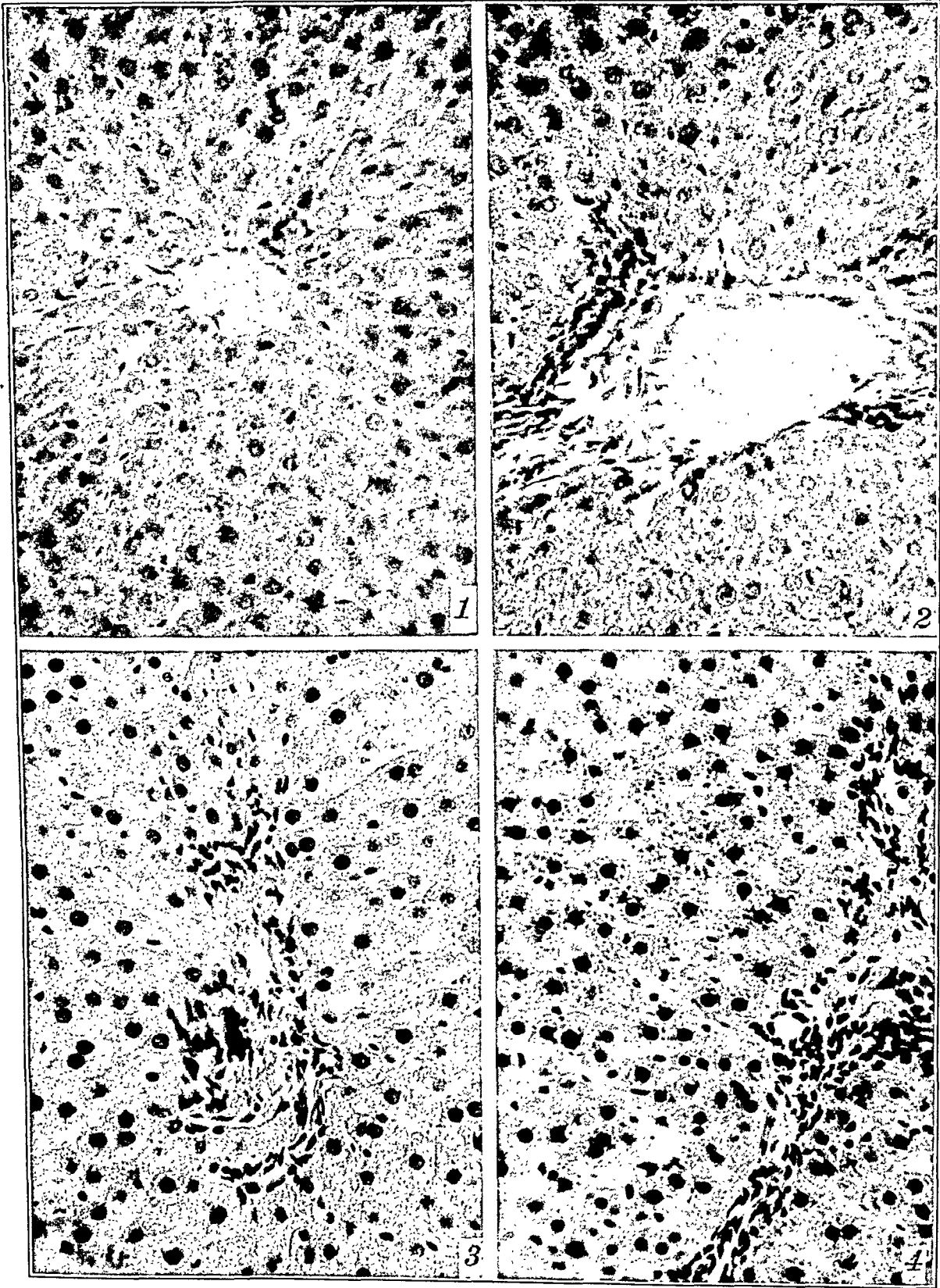


PLATE 72

- FIG. 5. Liver fixed 8 hours following an acute injection of morphine hydrochloride. This photomicrograph shows an extreme variation of the reaction of the mitochondria to the poison. The material was fixed in Regaud's fluid prior to staining with acid fuchsin and methyl green.
- FIG. 6. Depicting an extreme variation of mitochondrial behavior in the pancreas 5½ hours following an acute injection of the drug. Observe the formation of lightly staining globules and the absence of filamentous mitochondria. Material was fixed and stained as above.
- FIG. 7. Control section of pancreas from normal healthy rat fixed and stained as above. Note the mitochondrial filaments are restricted to the basal region of the acinar cells and also the absence of globules.

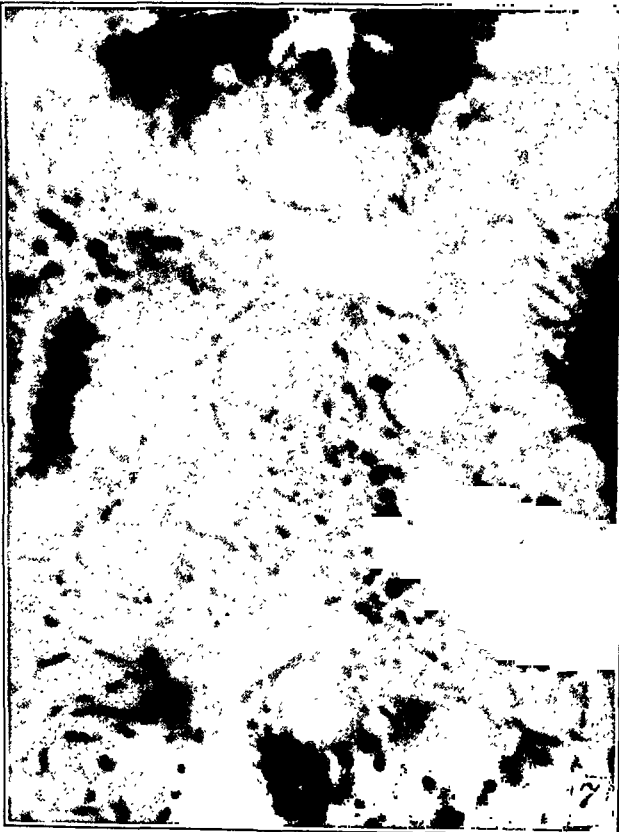
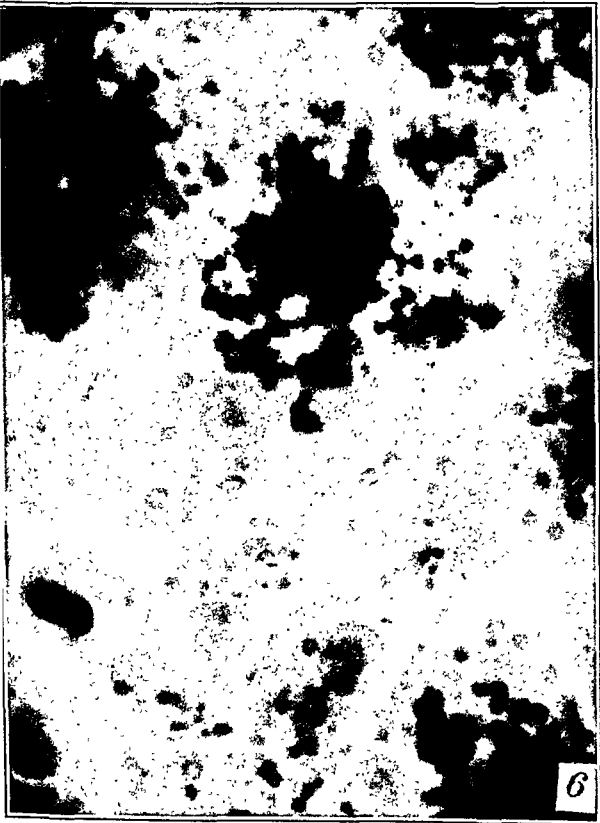
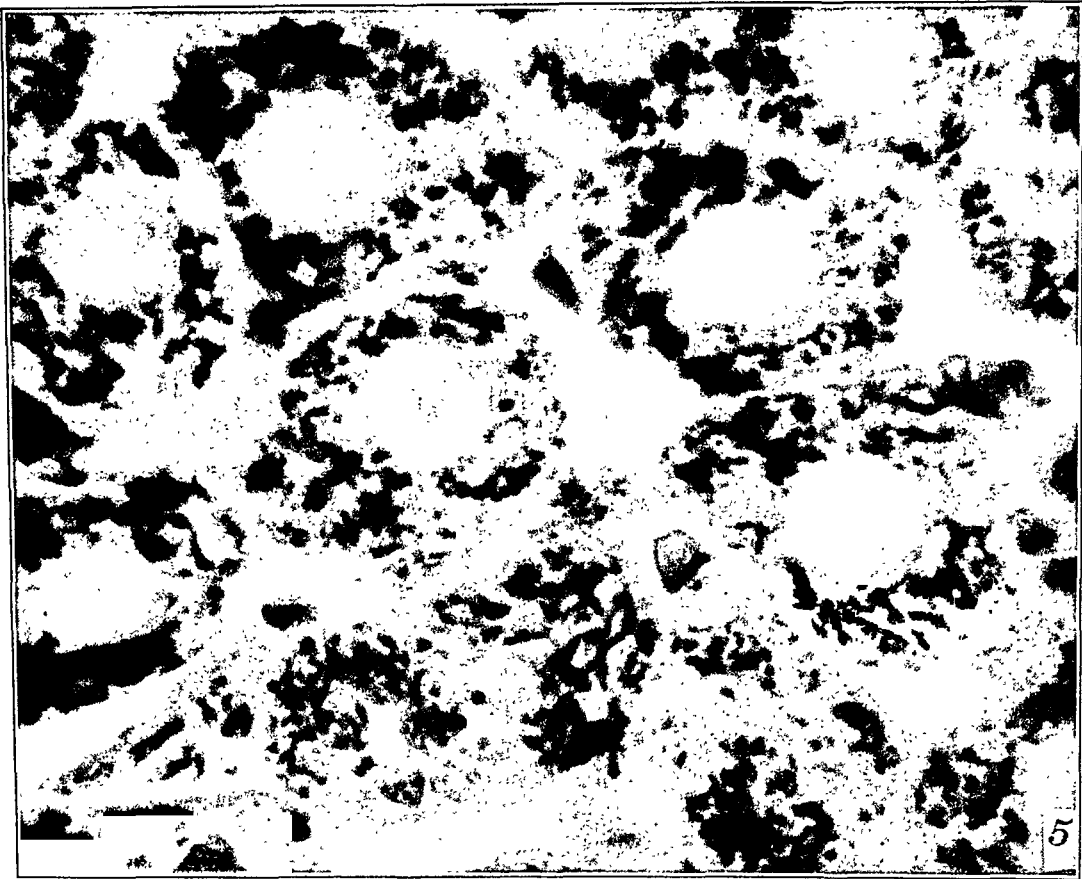


PLATE 73

FIG. 8. Showing filamentous structure of mitochondria as seen in the absorbing columnar epithelium of the intestinal villi in a normal rat after fixation in Regaud's fluid and staining by acid fuchsin and methyl green. Note the longitudinal arrangement of mitochondria to the axis of the cell, together with the basal or subnuclear aggregation of mitochondria forming a capsule around the nucleus.

FIG. 9. Demonstrating the reaction of mitochondria to chronic poisoning in the epithelium of the intestine of a 6½ months morphine addict. The mitochondria have a slightly depleted appearance in contrast with control in Fig. 8. Material fixed and stained by Ludford's variation of the Nassonov technique.

FIGS. 10 and 11. Revealing an extreme reaction of mitochondria to a single massive injection of morphine hydrochloride. In this material the mitochondria were seen to vary from chainlets to isolated spherical bodies within the same preparation. In these figures the mitochondria are seen as rounded hypertrophied structures. Compare with control formation in Fig. 8 and the chronic condition as demonstrated in Fig. 9. Material fixed and stained as in the latter.

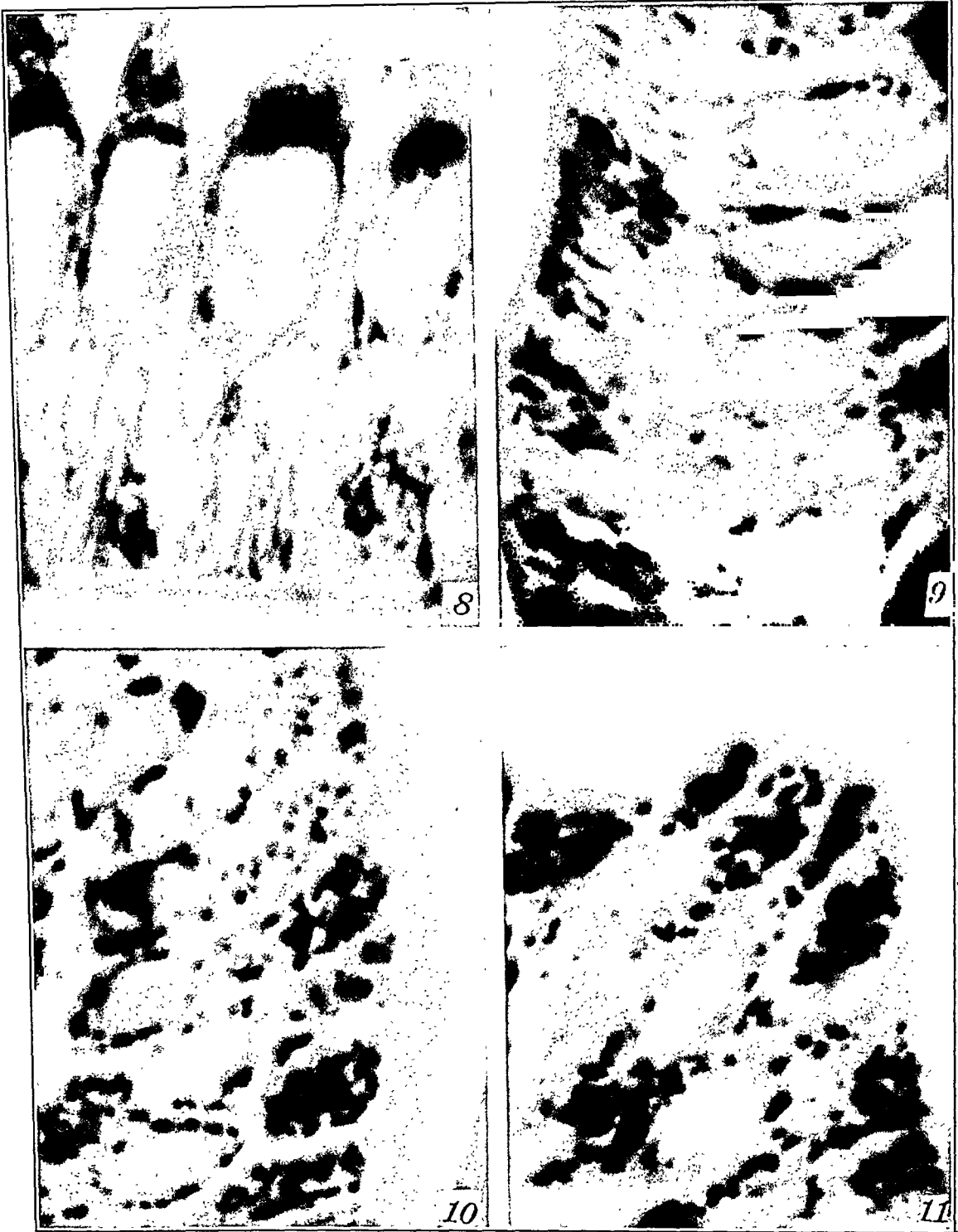


PLATE 74

- FIG. 12. Transverse oblique section through gastric gland of stomach in rat killed 7 hours following a single large dose of morphine. Observe slight hypertrophy and fragmentation of Golgi bodies. Material treated by Hirschler's modification (1918).
- FIG. 13. Showing depleted appearance of the Golgi substance in a transverse section of a duodenal gland of Brunner in the submucosa, obtained from a rat of 7 months addiction before withdrawal of the morphine. This photomicrograph shows an extreme variation of this condition and contrasts with the condition of the Golgi apparatus of acutely morphinized material, as seen in Fig. 12. Material fixed by Ludford's variation of the Nasonov technique counterstained by orange G in absolute alcohol.
- FIG. 14. Section cut transversely through a gland of Brunner of a normal lecithin-fed rat, killed during secretory activity. The Golgi bodies have become reduced, the mitochondria are granular and have migrated together with secretory droplets toward the apical region of the cell; this phenomenon is not due to lecithin feeding, and demonstrates the cytological state of a gland during normal functional behavior. Tissue fixed by Ludford's variation of Nasonov's technique, counterstained with orange G.
- FIG. 15. Showing the cytological appearance of a single gastric gland of a lecithin-fed rat of 6 months addiction, killed 4 days after abrupt withdrawal of morphine. The Golgi material is rendered clearly in the apical region of each cell in the gland. The Golgi substance still retains its depleted appearance peculiar to the chronic condition, and has not responded to lecithin administration. Tissue fixed and stained as in Fig. 14.

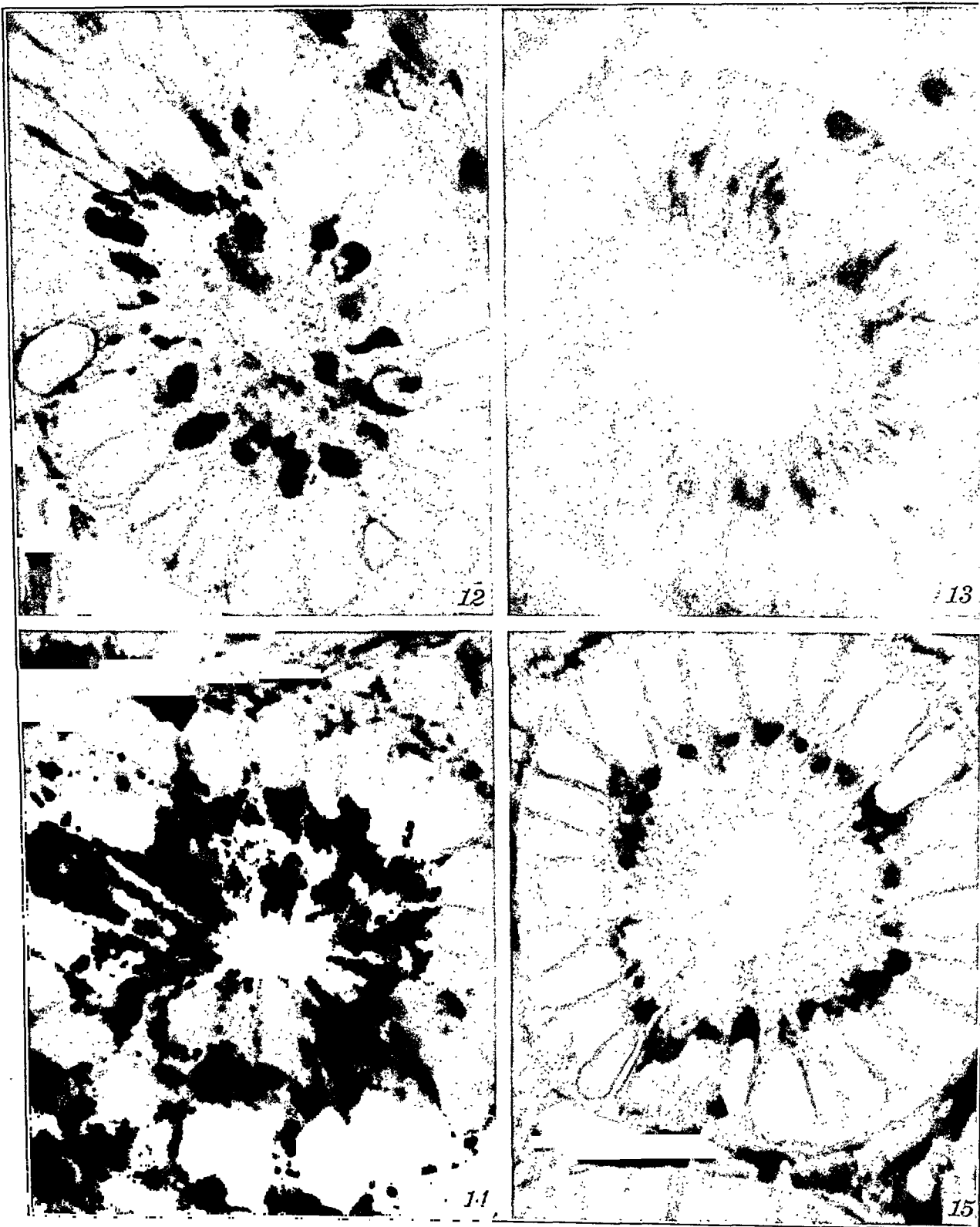


PLATE 75

FIGS. 16, 17 and 18. Revealing the structure of the Golgi material in nerve cells of spinal ganglia of normal healthy rodents. Observe the diverse morphology of the apparatus in the larger and smaller cells. In the former the Golgi apparatus is dispersed, forming a broken network throughout the cytosome (Fig. 18). The Golgi substance in the smaller cells forms a compact mass around the nuclear membrane (Fig. 16). This material was treated by Ludford's variation of Nasonov's Golgi technique and stained by orange G in 95 per cent alcohol.

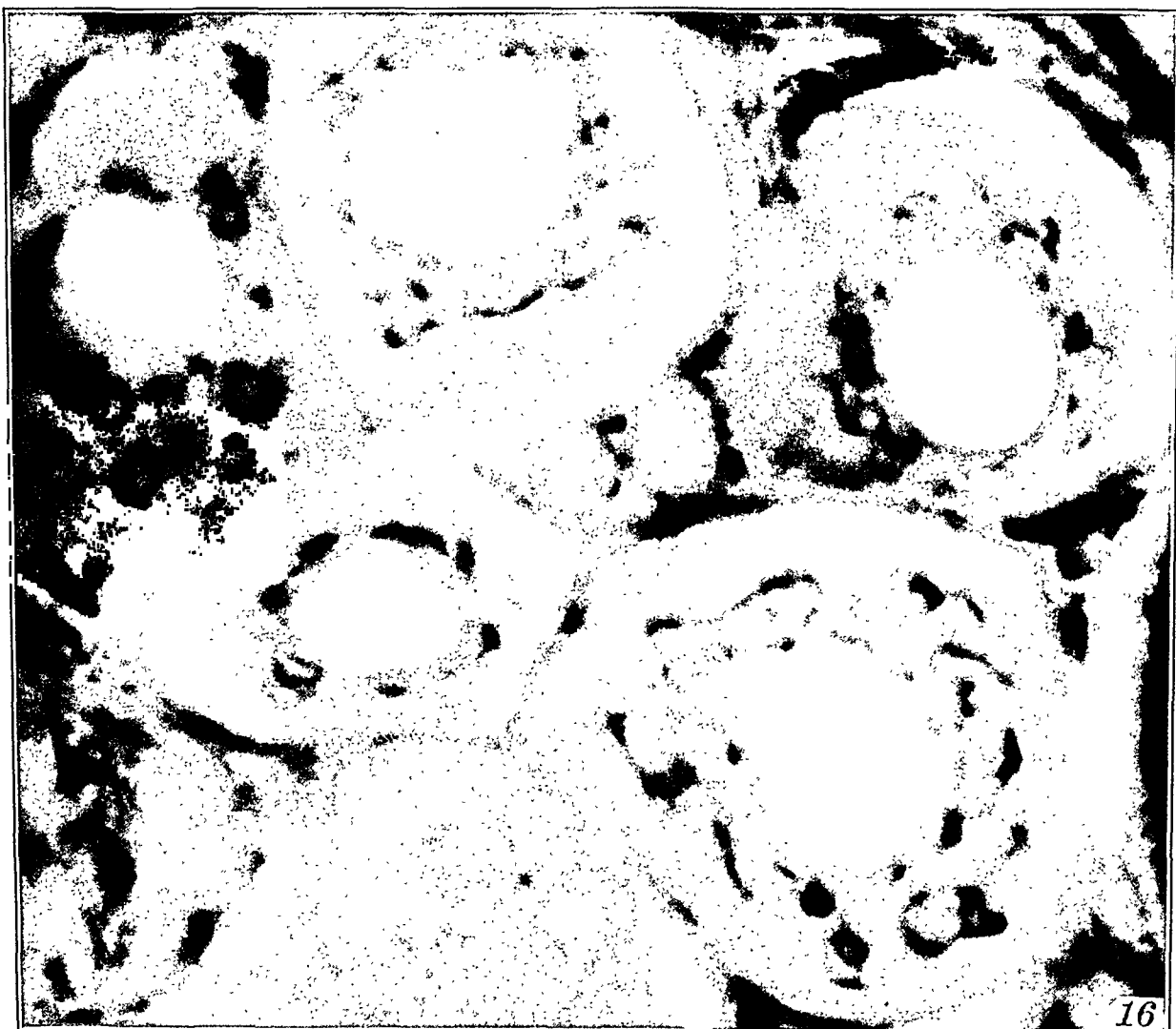
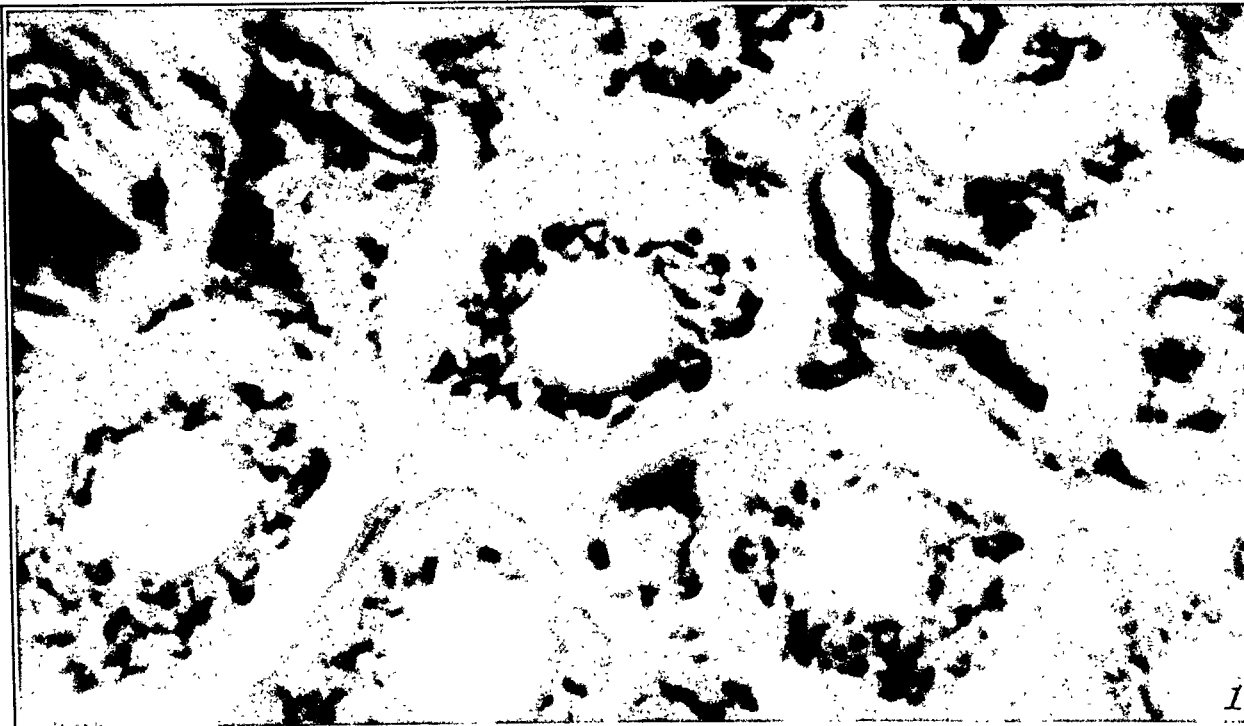


PLATE 76

FIGS. 19, 20 and 21. Depicting nerve cells of spinal ganglia in a rat following massive injection of morphine hydrochloride, obtained at intervals from 3 to 5 hours after administration of the drug. Fig. 19 shows the condition of the Golgi apparatus 3 hours after injection. In both larger and smaller cells the Golgi material has undergone a slight fragmentation and hypertrophy, which is apparent when compared with the appearance of the apparatus in normal material (see Figs. 16, 17 and 18). Figs. 20 and 21 represent material taken 5 hours after a like dose in which the Golgi apparatus presents a similar appearance to that seen in Fig. 19. In Fig. 20 the apparatus in the smaller cells has undergone less fragmentation than in the larger, but displays a reaction to the drug in the form of a slight hypertrophy.



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HISTOLOGY OF THE CORONARY ARTERIES AND THEIR BRANCHES IN THE HUMAN HEART *

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The descriptive literature on the coronary arteries and their branches is remarkable for its paucity. This is all the more extraordinary in view of the fact that diseases of the coronary arteries are occupying more attention today than ever before. With the exceptions of the reports by Wolkoff,¹ and Ehrich, de la Chapelle and Cohn,² few significant contributions have been made to this field during the last 15 years. This fact, together with a need for an accurate description of the age period changes in the finer myocardial vascular channels, has led us to report our investigations in this field. It seemed worth while to make a systematic histological study of the main coronary vessels and myocardial twigs, ranging in caliber from moderate sized branches down to capillaries, in order to determine their normal structure as represented in the first eight decades of life, and to observe whether or not there were any significant differences in the myocardial vessels in the various parts of the heart. Our observations, in common with others, have shown that the variations in structure of the coronary branches are not inconsiderable, even for the same age period. Since the reported observations are based largely on a study of relatively few normal hearts and because of the difficulty in obtaining material that would give a fair representation of what might be called the normal coronary vessel, these studies were made on fifty carefully chosen specimens. Diseases that were likely to implicate the coronary vessels were excluded, as were those hearts that showed obviously diseased coronary arteries on gross inspection.

METHODS AND MATERIALS

The hearts were fixed in 10 per cent formalin saline † and blocks cut according to the standardized method of Gross, Antopol and Sacks.³ By this technique

* Aided by a grant from the Lucius N. Littauer Fund.

† Solution of formaldehyde U. S. P. 10 parts, 1 per cent sodium chloride solution 90 parts. This solution is rendered neutral with a weak alkali.

Received for publication September 20, 1933.

six blocks are obtained representing those areas of the heart that experience has shown are most likely to present pathological changes. They can reasonably be called strategic areas in the heart. Together with these six blocks there was also removed one block from the left circumflex coronary artery, 1 cm. from its ostium; one from the left anterior descending branch, 2 cm. from the ostium of the left circumflex coronary artery; one from the right circumflex coronary artery, 1 cm. from its ostium; and one from the posterior descending branch, 0.5 cm. below the auriculoventricular sulcus. The blocks were dehydrated and infiltrated in the usual manner. In the later age periods it was occasionally necessary to decalcify the specimen. Seven and one-half micron sections were cut from each block and stained as a routine with hematoxylin and eosin and Weigert's elastic and Van Gieson's connective tissue stains. Since we were primarily interested in tissue changes rather than infiltrations, relatively few fat stains were done.

GENERAL CONSIDERATIONS

In order that the nature of our material may be understood more clearly it is necessary to define our conception of what, for purposes of this report, constitutes a normal vessel. As is well known, certain progressive changes, particularly affecting the intima of the coronary arteries, appear soon after birth. These changes consist of splitting of the lamella elastica interna, the appearance of muscular elements between these split layers (the so-called musculo-elastic layer *) and the further development of elastic and connective tissue elements internally to the latter. It is the opinion of several authors that some of these changes (development of elastic-hyperplastic and connective tissue layers) already constitute early stages of sclerosis, particularly since fatty changes not infrequently occur concomitantly with them. Whether these uniformly occurring phenomena should be placed under the category of pathological processes becomes a matter of definition.

In order to avoid confusion and to simplify the problem we shall not attempt to make this differentiation, since it would lead us into the rather philosophical question as to whether or not the occurrence of pathological changes can be considered a normal process, and to the more theoretical question as to what constitutes a pathological change. It will suffice us to consider the development of these internal layers as normal and to exclude as abnormal the deposition and formation in them in appreciable amounts of such addi-

* We prefer the term "musculo-elastic layer" to "elastic-muscular layer."

tional elements as calcium salts, lipoid crystals, blood vessels and cells of inflammation.

While such a classification as to the normality of a blood vessel is arbitrary, it has the advantage that one must select as a base line such material as shows the least departure from the simple structure represented in the newborn. It also obviates the necessity of including such conspicuous changes as can legitimately be considered under the category of atherosclerosis. Limiting our material in this manner makes it increasingly difficult to find normal vessels in the later age periods. However, with careful selection cases can be found that show a minimum of such changes, even in quite advanced age periods.

In order to define more accurately the problems to which particular consideration was given in these studies it might be well to give first a brief description of the main coronary artery structure which represents a cross-section of opinions gathered from the works of Colucci,⁴ Edholm,⁵ Faber,⁶ Jores,⁷ Wolkoff,¹ Bork,⁸ Ehrich, de la Chapelle and Cohn,² and Spalteholz and Hochrein.⁹ This will be followed by a brief description of the small myocardial twigs, based on Wolkoff's description.

According to present conceptions the larger coronary vessel consists of three main layers, adventitia, media and intima. The adventitia is made up of a meshwork of connective tissue whose density increases with age. Particularly in the inner layers of the adventitia are to be found elastic fibers running largely in a circular direction. The media consists of smooth muscle, for the most part circular in arrangement. Scattered among the smooth muscle fibers are circularly arranged elastic elements that are generally more conspicuous toward the outer layers of the media. The intima consists at birth of a single elastic lamella (*lamella elastica interna*) covered with flat endothelium. With increasing age the intima becomes more complex, this complexity being visible chiefly in the larger vessels and main trunks. The first change consists of splitting of the *lamella elastica interna* into two membranes between which smooth muscle fibers appear, running at times diagonally but generally in a longitudinal direction. This constitutes the musculo-elastic layer. The outermost of these elastic membranes continues to represent the border line between intima and media and accordingly retains the name *lamella elastica interna*. The innermost

layer, as will be seen, undergoes numerous changes in addition and is referred to as the "inner limiting membrane." Because of the confusion of this term with "lamella elastica interna" we prefer to designate it "secondary intimal elastic membrane." Fine longitudinal elastic fibers are found between the smooth muscle cells of the musculo-elastic layer. Already in the earlier age periods breaks in continuity begin to appear in the lamella elastica interna. Through these breaks smooth muscle cells from the media may be seen pressing themselves into the musculo-elastic layer. On the other hand, by a process of splitting of the secondary intimal elastic membrane, the elastic-hyperplastic layer is formed. This layer takes on great proportions with developing age. Finally, the inner portions of this layer develop a preponderance of connective tissue elements (collagen) and form the so-called connective tissue layer. These last three mentioned subdivisions of the intima increase in thickness with age until the intima eventually becomes considerably thicker than the media. Simultaneously, however, with the development of these layers, areas of discontinuity appear within them so that at times it is difficult to distinguish their limiting borders. In places, one or another of these layers may be missing. In later age periods calcific and atherosclerotic changes occur in the intima.

According to Wolkoff the adventitia of the small myocardial twigs consists of a wide connective tissue feltwork intermingled with elastic tissue which is more concentrated toward the media but forms no definite external elastic lamella. The media consists of circular smooth muscle and elastic fibers. The intima is made up of flat endothelium resting on a lamella elastica interna consisting of anastomosing longitudinal bands of elastic tissue. Wolkoff observed no age period changes in these vessels.

In analyzing our material an attempt will be made to follow the evolution of each main layer from birth to the eighth decade, giving thus a dynamic picture of the changes that take place. Since our observations indicate that by no means do the age period changes in some of the smaller vessels occur, so to speak, at the same tempo in the various parts of the myocardium, an attempt will be made to point out these differences. It will be seen that to discuss the extent of progressive changes in the so-called myocardial arteries is of relatively little value unless indication is given as to the location of the vessel in the heart.

It is to be borne in mind that the description must of necessity be one representing the average of a number of preparations, that very wide variations occur in the different parts of the same vessel, and that a given change is seldom uniform even throughout one microscopic section. In particular, the progressive changes in the intima are unevenly distributed so that it is thrown up into irregular plateaus, and our discussion will represent the average process taking place in a given section.

Because of the wide variations in individual vessels much of the published matter loses considerable significance as the individual variations cannot be considered along statistical lines. A notable exception to this is the report of Ehrich, de la Chapelle and Cohn.² In our studies an attempt was made to examine considerable material in the very early age periods, as this has received scant attention. Our description, therefore, will be based on the sequence of events as they are found month by month in the earliest age periods and within very close periods of time thereafter until the end of the first decade. An interesting chapter in the discussion of coronary artery structure, to which apparently little or no attention has been paid in the past, will be the description of the histology of the myocardial twigs, arterioles and capillaries.

LEFT CIRCUMFLEX CORONARY ARTERY

Intima: In the first two months of life the intima of this vessel is confined practically to a single layer of flat endothelium which rests on a single continuous lamella elastica interna. As early as the third month this elastic membrane may begin to show areas in which splitting has taken place. These split areas enclose for the most part longitudinal fibers of smooth muscle (Fig. 1).

From this time on the splits become more extensive and conspicuous, so that by the end of the first year one not infrequently encounters specimens in which the entire circumference of the vessel shows a complete doubling of the lamella elastica interna. In such cases the outermost elastic layer (the lamella elastica interna proper) is generally thick and more or less continuous on cross-section, the innermost (secondary intimal elastic membrane) is often more delicate, may be discontinuous and shows many of its elastic fibers running in a longitudinal direction. The smooth muscle between these layers

increases in bulk and often shows delicate longitudinal elastic fibers running between them (the musculo-elastic layer) (Fig. 2).

Simultaneously one also begins to encounter a series of progressive changes in the intima which can be stated briefly as represented by the appearance of discontinuities in the lamella elastica interna (already seen in the third month) and continued splitting of the secondary intimal elastic membrane (generally longitudinal elastic fibers) with the formation of connective tissue between the elastic fibers constituting the so-called elastic-hyperplastic layer (end of the first year).

Wherever discontinuities appear in the lamella elastica interna the musculo-elastic layer lies in intimate relation to the subjacent media. It will be shown later that in such underlying layers of the media the smooth muscle cells may also run in a longitudinal direction, with the result that it becomes impossible sharply to delimit media from intima (Figs. 3 and 6). This will be termed "border disappearance" and the longitudinal smooth muscle layer occupying these areas will be referred to as the "intermediary layer." The frequency with which this intermediary layer is found will be taken up under the description of the media.

By the end of the second year the development of the elastic-hyperplastic layer has become quite conspicuous. The intima at this time, however, with few exceptions, still remains considerably narrower than the media. The above described changes, namely, proliferation of the elastic-hyperplastic layer, growth of the musculo-elastic layer and appearance of discontinuities in the lamella elastica interna, with development of the intermediary layer, continue progressively and are quite well established by the end of the first decade.

During the second decade the musculo-elastic layer becomes prominent and border disappearance is quite regularly met with. With the development of the elastic-hyperplastic layer even in the early age periods (end of the first year) the simple schema of structure for the musculo-elastic layer becomes altered in several respects. First, the smooth muscle elements are no longer confined between two elastic membranes but can be seen spreading irregularly so that they are found encroaching on the elastic-hyperplastic layer. They also occur irregularly distributed throughout the circumference of the intima, appearing in greater concentration in some areas and in

lesser concentration in others. It is, therefore, generally difficult to speak of a sharply defined musculo-elastic layer after the first five years of life.

During the second decade the width of the intima generally equals from one-half to three-quarters that of the media, although in places it may exceed the latter. One also encounters occasionally a transformation of the elastic-hyperplastic layer into dense collagenous tissue, thereby forming the connective tissue layer. This transformation is met with somewhat more frequently during the third decade but does not become a regular constituent of the intima until the fourth decade. Meanwhile, during the third decade the intima not infrequently equals or exceeds the media in width.

From this time on the intima continues to widen, so that by the end of the fifth and the beginning of the sixth decade it is generally several times the thickness of the media. With the appearance of consistently well marked connective tissue layers one not infrequently encounters the deposition of calcium salts and lipoid crystals in the latter. Quite apart from this, however, from the fifth decade on one begins to note encroachment of the thickened intima on the media. In such cases one may see a decrease in the muscular elements of the musculo-elastic layer. These changes occur in exaggerated form during the sixth, seventh and eighth decades (Fig. 4).

Media: The media of the left circumflex coronary artery in its simplest form consists of a band of circularly arranged smooth muscle intermingled with elastic fibers which run for the most part also in a circular direction. These elastic fibers are irregular in their distribution. They occasionally appear in greater concentration toward the inner half of the media but are usually more conspicuous toward the outer half (Fig. 5). The quantity of elastic fibers in the media seems to increase somewhat with age. In those areas of the media that lie immediately beneath a thickened portion of the intima, especially if the latter encroaches on it, the elastic fibers are generally more prominent.

In the second month of life one begins to note bundles of longitudinal smooth muscle in the media which are generally situated immediately beneath the intima (Fig. 5), but are occasionally seen lying more deeply. In some areas these longitudinal smooth muscle bundles, interspersed with longitudinal elastic fibers, may become so extensive as to encircle the entire circumference of the vessel. In

such cases, however, there is almost invariably seen border disappearance, so that the longitudinal smooth muscle becomes a component of the intermediary layer (Figs. 3 and 6).

Toward the end of the first year irregular areas of longitudinal smooth muscle begin to appear somewhat more frequently. They occur with great regularity as a component of the intermediary layer during the second decade.

During the second year of life one begins to observe the presence of collagenous fibers in the media. These, however, do not increase conspicuously in quantity until the second decade. They become very prominent from the middle of the fourth decade on.

When sclerotic changes begin to appear in the intima there are seen frequently atrophic changes in the muscular elements of the media, with the encroaching elastic-hyperplastic intima dipping deeply into it. Vasa vasorum are not found normally in the media. With advancing atherosclerotic transformation, however, blood vessels may appear in the media and penetrate into the sclerotic intima.

Adventitia: This outermost supporting structure of the blood vessel consists at birth of relatively loose bundles of connective tissue running circularly and diagonally, interspersed with circular and longitudinal elastic fibers. The latter are so decidedly concentrated at the media-adventitia border that they may be considered to form a lamella elastica externa, even though this is not a continuous membrane. The thickness and quantity of these elastic fibers increase rapidly during the first year of life. The increase thereafter until the end of the first decade is proportionate to the growth of the vessel. From then on the fibers continue to concentrate immediately outside the media-adventitia border, but decrease in quantity throughout the rest of the adventitia.

The connective tissue fibers become progressively more compact from birth to the end of the eighth decade.

Review of Characteristic Histological Features of the Left Circumflex Coronary Artery

In reviewing these findings in the left circumflex coronary artery certain points may be emphasized as follows:

1. The early splitting of the lamella elastica interna.

2. The rapid formation of the elastic-hyperplastic layer.
3. The diffuse and irregular growth of the musculo-elastic layer, which soon passes internally beyond the border of the so-called secondary intimal elastic membrane.
4. The rapid loss in continuity of the secondary intimal elastic membrane.
5. Existence of longitudinal smooth muscle in the media.
6. The formation of the intermediary layer by fusion of the musculo-elastic layer with smooth muscle of the media. This occurs particularly after the first decade.
7. The rapid concentration of elastic fibers in the adventitia in the zone immediately external to the media, with the disappearance of these fibers from the rest of the adventitia.

A comparison of the development of these structures, as between the various coronary arteries, will be discussed following the description of each vessel.

LEFT ANTERIOR DESCENDING BRANCH

Intima: This vessel generally differs from the other coronary branches in that its intima appears to be considerably advanced in progressive changes over that of the left circumflex coronary artery. At birth one can see irregular elastic-hyperplastic thickenings of the intima, fine splitting of the lamella elastica interna and early formation of the musculo-elastic layer. By the second month of life discontinuities are noticed in the lamella elastica interna with here and there the formation of the intermediary layer in areas of border disappearance. Toward the end of the first year of life the intima may equal the width of the media in places. This is not found with any degree of regularity, however, until the end of the first decade. By the fifteenth year border disappearance may have become so complete that the intermediary layer occasionally occupies the entire circumference of the vessel. By the end of the second decade the intima is often twice the thickness of the media. This thickening, largely due to the growth of the elastic-hyperplastic layer, continues rapidly so that by the fourth decade it is frequently several times the width of the media.

A somewhat less obvious difference in the development of the intima in the left anterior descending branch, as compared with that

of the left circumflex coronary artery, is the appearance of the connective tissue layer somewhat earlier, namely, during the end of the third decade, although its regular appearance is not encountered until the fourth decade.

The orderly arrangement of the musculo-elastic layer, which is already well established during the middle of the first year, is lost much earlier in the left anterior descending branch than in the other vessels. From the second year of life on one frequently encounters the musculo-elastic layer so advanced in development that it spreads within the elastic-hyperplastic zone.

One of the most important characteristics of the left anterior descending branch is the appearance of lipoid and calcific deposits more frequently, more conspicuously and earlier in this vessel than in the other coronary branches. As a consequence encroachment of the intima on the media is met with very frequently from the fourth decade on. After the fifth decade the intima is rarely encountered without lipoid and calcific deposits.

Media: The media of the left anterior descending branch shows no conspicuous differences from that of the left circumflex coronary artery until the latter half of the second year of life when the more frequent occurrence of the intermediary layer alters its characteristic structure. As in the left circumflex coronary artery, the growth of the media on the whole parallels that of the myocardium. The elastic fibers which are generally richer in the left anterior descending branch than in the other coronary branches increase slightly in quantity with age until the first decade. From this time on they seem to diminish abruptly in quantity, except in those areas underlying sclerotic plaques, as mentioned in the left circumflex coronary artery description. The distribution of the elastic fibers in this vessel is also generally more toward the outer half of the media. The collagen fiber component of the media can be seen well during the second year of life. Its increase, however, is not rapid. It becomes conspicuous during the third decade.

Adventitia: The structure and development of the adventitia of this vessel is similar to that described for the left circumflex coronary artery. Its width, however, is generally greater than that of the other coronary branches. Decrease in the extent of elastic fibers distributed throughout the adventitia occurs from the end of the first decade on, as found in the left circumflex coronary artery.

Review of Characteristic Histological Features of the Left Anterior Descending Branch

The important points to be emphasized as peculiar to the left anterior descending branch are:

1. Earlier appearance of the elastic-hyperplastic layer.
2. Rapid progress in the development of the elastic-hyperplastic layer with marked thickness of the intima.
3. Earlier appearance and more marked deposits of lipid crystals and calcium salts in the intima.
4. More rapid development and spread of the musculo-elastic layer.
5. Earlier and more frequent occurrence of the intermediary layer.
6. Somewhat greater width of the adventitia.

RIGHT CIRCUMFLEX CORONARY ARTERY

Intima: On the whole the intima of this vessel maintains its simplicity of structure for a longer period of time than that of the left anterior descending branch or the left circumflex coronary artery. While minute splittings of the lamella elastica interna can at times be detected in the seventh week of life this does not become conspicuous until the latter part of the first year, at which time the musculo-elastic layer also begins to make its appearance. Elastic-hyperplastic changes appear with greater regularity by the end of the eighteenth month and discontinuities of the lamella elastica interna, which are rare up to the end of the second year, also begin to appear more frequently at this time.

The continued growth of the intima differs from that of the left circumflex coronary artery and the left anterior descending branch in several respects. The musculo-elastic layer is generally narrower than in the other vessels. The rapidly and earlier developing connective tissue layer encroaches on the musculo-elastic layer, producing atrophy of its smooth muscle fibers. Connective tissue changes in the elastic-hyperplastic layer can sometimes be seen in the second half of the first decade. They are encountered with considerable regularity during the third decade and thereafter develop more frequently and rapidly than in the other coronary vessels. Indeed,

the connective tissue layer is generally more conspicuous in this vessel during the later decades than is the elastic-hyperplastic layer.

It has been mentioned that the lamella elastica interna discontinuities begin to appear with some degree of frequency after the second year of life. On the whole, however, they are generally less marked in this vessel. This is also true for the incidence of border disappearance.

The intima is generally half as wide as the media during the second decade and equals it during the third decade. During the fourth decade and thereafter, however, it generally becomes very wide because of the considerable development of the connective tissue layer.

Media: The media of the right circumflex coronary artery corresponds in development very closely to that of the left circumflex coronary artery with the following sharp differences. (1) The intermediary layer is encountered with less frequency, as are the longitudinal smooth muscle bundles elsewhere in the media, and (2) the connective tissue elements (collagen fibers) appear much earlier. With respect to the latter it may be said that even as early as toward the latter half of the first decade they are seen with a fair degree of regularity. They rapidly become more conspicuous from the second decade on. The quantity and distribution of elastic fibers in the media are similar to that found in the left circumflex coronary artery.

Adventitia: The adventitia of the right circumflex coronary artery shows in its connective tissue elements a type of development that is similar to the two vessels previously described. Its width is generally similar to that of the left circumflex coronary artery and the elastic fibers behave very much like those of the adventitia of the left circumflex coronary artery in respect to concentration.

Review of Characteristic Histological Features of the Right Circumflex Coronary Artery

The important points to be emphasized as peculiar to the right circumflex coronary artery are:

1. Later appearance of splitting of the lamella elastica interna.
2. Generally narrower width of the musculo-elastic layer and its later atrophy, because of the considerable development of the connective tissue layer in the intima.

3. The later and less marked discontinuities in the lamella elastica interna.
4. Earlier and considerable development of the connective tissue layer.
5. The growth in width of the intima as a whole is similar to that of the left circumflex coronary artery.
6. Less frequent occurrence of border disappearance and the intermediary layer.
7. Earlier appearance of collagen fibers in the media.

POSTERIOR DESCENDING BRANCH

Intima: In describing the histology of the posterior descending branch one must bear in mind the fact that the structure varies considerably, depending on whether the posterior descending branch is large or small. This variation is much greater for the posterior descending branch than for the other coronary vessels. If the vessel is large its structure and age period changes will approximate those described for the right circumflex coronary artery. Generally, however, the vessel is considerably smaller, in which case the intima maintains its simple form for a much longer period of time.

Assuming the smaller form to be the more frequent one may say that occasionally minute splittings of the lamella elastica interna with development of longitudinal smooth muscle fibers may be encountered during the first year of life but do not become very marked until the latter half of the first decade. Elastic-hyperplastic changes are very scant until the middle of the second decade, at which time the lamella elastica interna discontinuities also begin to appear with some degree of regularity.

The intima reaches in width half that of the media, generally during the third decade. Toward the end of the fourth decade it equals the media in width but grows rather slowly. Occasionally, during the fourth decade, irregular thickenings many times the width of the media may be found. The connective tissue layer generally does not make its appearance until the fifth decade. In keeping with the slower tempo of changes in the posterior descending branch lamella elastica interna discontinuities are encountered much less frequently, as are border disappearances and the development of the intermediary layer. The latter occurs very irregularly.

It follows from this description that sclerotic changes in the posterior descending branch make their appearance later in life than in the other main vessels.

Media: The media of the posterior descending branch shows the least amount of elastic tissue of the four vessels described. These fibers tend to be more concentrated toward the external half of this layer and generally disappear practically completely after the first decade. On the other hand, the posterior descending branch resembles the right circumflex coronary artery in the earlier appearance of connective tissue elements, which are fairly well discernible toward the end of the first decade and become more conspicuous thereafter.

Adventitia: The adventitia of the posterior descending branch is narrower than in the other vessels described. At times, however, it may be considerably wider than the others. Its elastic fibers are generally fairly sparse, increase somewhat from the second year of life to the end of the fifth year of life, and decrease again thereafter.

Review of Characteristic Histological Features of the Posterior Descending Branch

The important points to be emphasized as peculiar to the posterior descending branch when it occurs as a small vessel are:

1. Very late appearance of splitting of the lamella elastica interna and development of the musculo-elastic layer.
2. Very late occurrence of discontinuities in the lamella elastica interna. These are rather inconspicuous.
3. Very late appearance of elastic-hyperplastic changes.
4. Very late appearance of the connective tissue layer.
5. Very slow increase in the thickness of the intima as a whole.
6. Late appearance of lipoid and calcific deposits.
7. Very infrequent occurrence of border disappearance and inter-mediary layer.
8. Scant elastic fibers in the media and their early disappearance.
9. Generally narrower adventitia.

MYOCARDIAL VESSELS

The coronary artery branches within the myocardium will be described under the categories of myocardial arteries, arterioles and capillaries. Each vessel will be considered in respect to age period

changes and location within the myocardium, based on an examination of the standardized blocks. It will be seen that of these three types of vessels the myocardial artery is the only one showing marked differences in structure and evolution dependent on its site within the heart.

Myocardial Arteries: Under this category are considered those vessels that possess three definite coats with a media consisting of more than one layer of circular smooth muscle fibers. Even in the earliest age periods one can already note remarkable differences between the medium sized twigs found in the various parts of the heart. The general arrangement of the component layers of these vessels is as follows. The adventitia is made up of a loose feltwork of connective tissue fibrillae with very sparse and delicate elastic fibers, both elements running in all directions. It may be mentioned here that the adventitial elastic fibers of the myocardial arteries in the auricles tend to be somewhat more numerous than elsewhere in the heart. On cross-section the adventitia is generally oval, filling in the interstices of the myocardial bundles. Because of this the width of the adventitia varies considerably. The media is composed of several layers of circular smooth muscle. The lamella elastica interna is made up of longitudinal elastic fibers. This is covered by flat endothelium.

At birth one occasionally notes circular elastic fibers intermingled with the smooth muscle cells of the media in some of the vessels in the posterior papillary muscle of the left ventricle and in the interventricular septum. These elastic fibers tend to form sheaths around the muscle cells. The continued development of these elastic fibers in the media will be referred to as "elastification of the media." In the fifth year of life one begins to encounter splitting of the lamella elastica interna and elastification of the media somewhat more often in the interventricular septum, but particularly in the posterior papillary muscle of the left ventricle. By the tenth year of life elastification of the media has become even more marked in the posterior papillary muscle of the left ventricle but can also be seen at times in the interventricular septum, left ventricle and pulmonary conus. At the beginning of the third decade the vessels in the posterior papillary muscle of the left ventricle may show elastic-hyperplastic changes of the intima with discontinuities in the lamella elastica interna.

Toward the latter part of the third decade another phenomenon begins to appear, particularly in the posterior papillary muscle of the left ventricle, namely, fusion of the elastic-hyperplastic intima with the elastified media. This is due to the fact that the elastification of the media may have gone on so rapidly that it is no longer possible to distinguish sharply the elastic-hyperplastic changes in the intima from the heavily elastified media. Furthermore, the intima may show smooth muscle fibers within its substance running in a longitudinal direction. At the same time, irregular patches of connective tissue may be found in the media replacing smooth muscle, so that the continuity of the latter is lost (Fig. 7). These changes are found with increasing frequency toward the end of the fourth decade and thereafter in the posterior papillary muscle of the left ventricle.

In the fifth decade splitting of the intima begins to be encountered in the pulmonary conus and auricles. Further progressive changes in these two sites are rather slow to develop. In the auricles especially, they rarely reach stages beyond rather mild media elastification with some intimal elastic splitting. In the seventh and eighth decades many of the vessels lying in the posterior papillary muscle of the left ventricle, interventricular septum and left ventricle have lost so much of their smooth muscle substance, developed so many connective tissue fibers in the media and intima, and undergone so much elastification of both these layers that they have been largely converted into elastic or fibro-elastic tubes. The possible physiological significance of these changes will be discussed later.

Arterioles: The arterioles of the myocardium differ from the arteries in several important respects. Apart from being considerably narrower in diameter, they possess a media made up of only a single layer of smooth muscle, their lamella elastica interna presents a beaded appearance until the eighth decade of life and they may or may not possess an adventitia, depending on their location in the myocardium.

The intima consists of a single layer of flat endothelium resting on a lamella elastica interna. This elastic membrane is only faintly discernible during the first two months of life. During this period it is made up of anastomosing, very delicate longitudinal fibers giving a beaded appearance on cross-section. From the third month until the seventh decade the fibers of the lamella elastica interna undergo

a progressive thickening and fusion but almost invariably maintain their beaded appearance on cross-section. Splitting of this membrane may begin to take place during the sixth decade but seldom becomes marked (Fig. 8). The split areas generally enclose collagen fibers.

The single layer of smooth muscle in the media may occasionally undergo atrophy during the very late decades. The atrophy does not generally involve the entire circumference of the vessel.

Many of the arterioles within the myocardium are devoid of an appreciable adventitia. If an arteriole is situated within the adventitia of an artery or within a somewhat heavier trabeculum of connective tissue it may be considered to possess an adventitial coat of its own. This varies considerably in contour and often consists of an irregularly arranged meshwork of collagen fibers intermingled with scant elastic fibers running in all directions. After the second decade of life the elastic fibers become more discernible and there can often be seen a single delicate elastic membrane surrounding the adventitia and forming a lamella elastica externa. This is at times discernible as early as the end of the first year of life.

The only point of interest to note with respect to differences in arterioles according to their site within the heart is that the adventitia is apt to be larger, and richer in elastic fibers in the auricles.

Capillaries: At birth the capillaries consist of delicate tubes made up of flat endothelium resting on a hyaline basement membrane. By the fifteenth year of life the capillaries throughout the heart may show a delicate elastic lamella outside the basement membrane. This, however, does not become conspicuous until the beginning of the sixth decade. There are no conspicuous differences to be noted in capillary structure within the various parts of the heart.

DISCUSSION AND SUMMARY

There has been presented in this report a description of the main coronary arteries of the human heart as well as of the myocardial arteries, arterioles and capillaries, as represented in six different areas in the heart (standardized blocks). The description particularly concerns itself with the age period changes that these vessels undergo. It is demonstrated that the main coronary vessels present a succession of progressive changes in the intima, media and adventitia which may be briefly summarized as follows:

1. Splitting of the lamella elastica interna with the formation of the musculo-elastic layer and the secondary intimal elastic membrane.

2. Continued splitting of the secondary intimal elastic membrane to form the elastic-hyperplastic layer.

3. Irregular growth and spread of the musculo-elastic layer into the elastic-hyperplastic zone.

4. Collagenic transformation of the elastic-hyperplastic layer to form the connective tissue layer.

5. Calcific and lipoid deposits in the intima with pressure atrophy of the underlying media and frequent development in the latter areas of additional elastic fibers.

6. Participation of the media and intima in the formation of the musculo-elastic zones termed "intermediary layers."

7. Development of intermediary layers concomitantly with the appearance of discontinuities in the lamella elastica interna, a process termed "border disappearance."

8. Increase in elastic elements in the media during the early age periods with decrease thereafter, particularly in the posterior descending branch.

9. Occurrence of longitudinal smooth muscle bundles in the media; seen with greater frequency during the later age periods.

10. Development of collagen fibers in the media.

11. Condensation of elastic fibers in the adventitia to form a more or less recognizable lamella elastica externa with partial disappearance of these elastic fibers from the rest of the layer with age.

It is further demonstrated that the left anterior descending branch presents these changes (with the few noted exceptions) more frequently and earlier than the other main coronary vessels. The left circumflex coronary artery is next in order chronologically and in the frequency with which these changes occur. The right circumflex coronary artery follows closely the development of the left circumflex coronary artery but possesses peculiarities of its own. The posterior descending branch, especially when it occurs as a narrow calibered vessel, is the last to show the above-mentioned progressive changes. Variations in the tempo with which these phenomena occur are great and even after making a comparison of the several vessels with a knowledge of the age of the individual it may be difficult or, at times, impossible to distinguish definitely the

left circumflex coronary artery, left anterior descending branch and the right circumflex coronary artery from one another. The left anterior descending branch generally may be recognized, however, by its more advanced progressive changes, the posterior descending branch by its markedly retarded developmental changes.

Obviously, the main coronary arteries thicken and stiffen with age. It would seem that vasodilatory changes can occur under these circumstances with increasing difficulty. Whether the rapidly developing intermediary layer may serve as a compensatory factor, *e.g.*, increase the diameter of the vessel by contraction of its longitudinal smooth muscle elements, is a matter for conjecture.

The elastic-hyperplastic changes can safely be considered to show all gradations into the definitely atherosclerotic process.

In the description of the age period changes of the myocardial arteries it has been demonstrated that a curious transformation takes place with age, *i.e.*, elastification of the media, elastic-hyperplastic changes in the intima, fusion of these two layers, atrophy of the smooth muscle elements and development of irregular patches of connective tissue — in short, a fibro-elastic metamorphosis of these vessels. Of greater importance is the fact that this process occurs at different times in various parts of the myocardium. Thus, of the standardized blocks examined, these changes are found first and most frequently in the posterior papillary muscle of the left ventricle; next in the interventricular septum, left ventricle and pulmonary conus, in this order; last and least in the auricles. That such fibro-elastic transformation of these vessels renders them more or less passive, *i.e.*, less susceptible to vasomotor control, seems evident. Narrowing of the larger vessels leading to these areas assumes, therefore, a serious portent, inasmuch as the fibro-elastified vessels probably cannot undergo sufficient vasodilatory changes to compensate for the narrowing. The importance of such vasodilatory changes was emphasized by Smith¹⁰ in 1921. He was interested in the question as to whether or not vasodilatation can occur in the coronary arteries and their branches following the administration of nitrites. His experiments consisted of tying off the distal branches of the left circumflex coronary artery in dogs. As a result of this procedure cyanosis of various degrees was produced in the myocardium, indicative of impending infarction. Following the administration of nitrites he observed, in some of the dogs, dis-

appearance of the cyanosis. In other experiments he measured the blood flow from the same vessels before and after the administration of sodium nitrite. Again, he was able to show a vasodilatory effect in some dogs. Smith concluded that in some dogs there is a communication with adjacent vessels which dilate under the action of these drugs.

If there is a similar vasodilatory compensatory mechanism in the human coronary arteries, as seems likely from observations to be published later, it helps to throw further light on certain observations made by Gross. In 1921 he demonstrated the gradual progressive development with age of wide anastomotic channels, particularly in the septum. Commenting on this observation Marvin¹¹ disagreed with the fact and suggested that the allegedly greater elasticity of the younger vessels either prevented the injection medium, used by Gross for study of these vessels, from entering the vessels, or squeezed it out into the larger ones.

If the elasticity of the vessel in any way parallels its content of elastic tissue our observations are diametrically opposed to Marvin's suggestion — certainly for the first three decades of life before appreciable fibrotic changes have developed. On the contrary, their very elasticity (tonic) and passivity could serve as a stimulus for the development of compensatory dilatation in other vessels supplying the same areas. In this connection it is of interest to note that these fibro-elastic transformations occur most frequently and most markedly in those areas of the heart that are the most frequent sites of infarction.

Finally, it is demonstrated that the myocardial arterioles present the above-mentioned changes in far less marked form and that the capillaries present with age only irregularly the development of an elastic layer outside its basement membrane.

It is hoped that these findings may serve as a baseline for comparative studies on the vessels of the human heart in disease.

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DESCRIPTION OF PLATES

PLATE 77

FIG. 1. Left circumflex coronary artery. Age 3 months. High power. Cross-section. Weigert's elastic and Van Gieson's connective tissue stain.

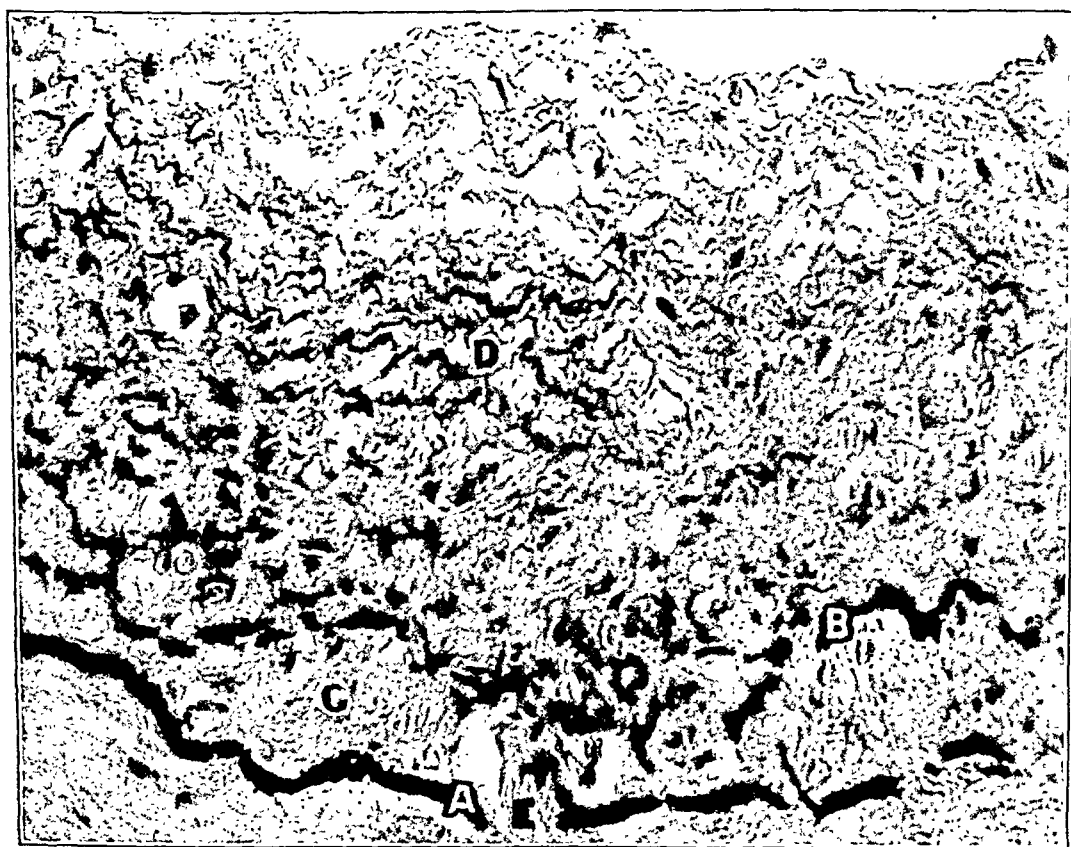
A = lamella elastica interna; B = secondary intimal elastic membrane; C = smooth muscle cell squeezing its way into intima from media through a discontinuity in the lamella elastica interna; D = media; E = adventitia.

FIG. 2. Left circumflex coronary artery. Age 5 years. High power. Cross-section. Weigert's elastic and Van Gieson's connective tissue stain.

A = lamella elastica interna, more or less continuous and heavy; B = secondary intimal elastic membrane, discontinuous, more delicate; C = musculo-elastic layer; D = elastic-hyperplastic layer; E = discontinuity in lamella elastica interna occupied by smooth muscle cell.



I



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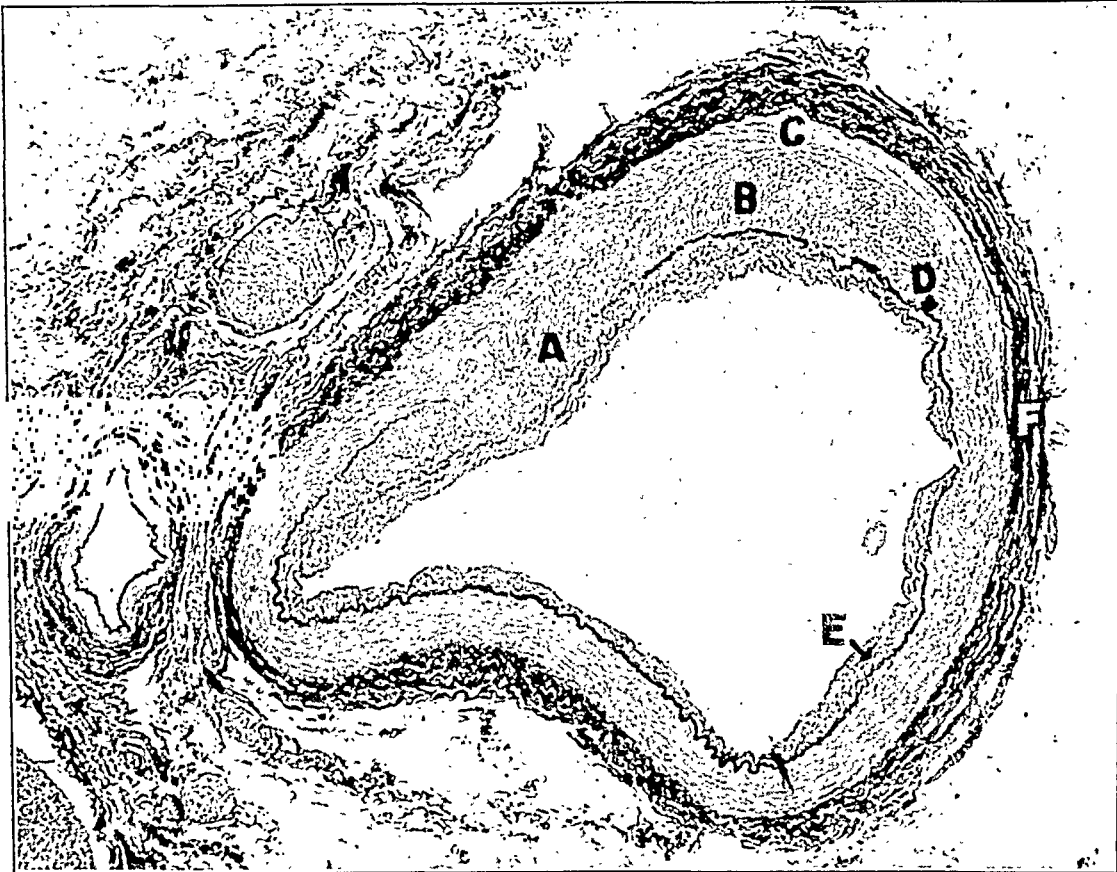
PLATE 78

FIG. 3. Left circumflex coronary artery. Age 18 months. Low power. Cross-section. Weigert's elastic and Van Gieson's connective tissue stain.

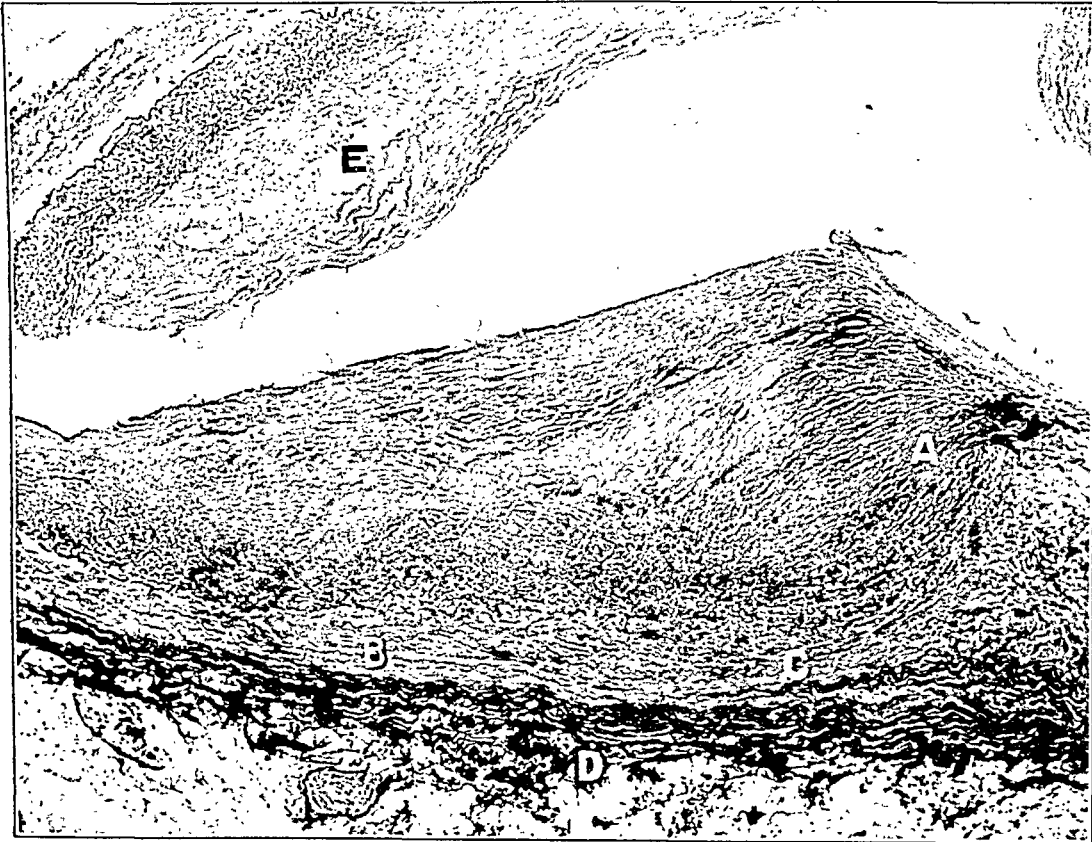
A = area of border disappearance occupied by intermediary layer; B = longitudinal smooth muscle in media; C = circular smooth muscle of media; D = lamella elastica interna; E = intima; F = adventitia.

FIG. 4. Left circumflex coronary artery. Age 74 years. Low power. Cross-section. Weigert's elastic and Van Gieson's connective tissue stain.

A = connective tissue layer in intima; B = media; C = atrophic portion of media heavily elastified; D = adventitia; E = lipoid and calcific changes in intima.



3



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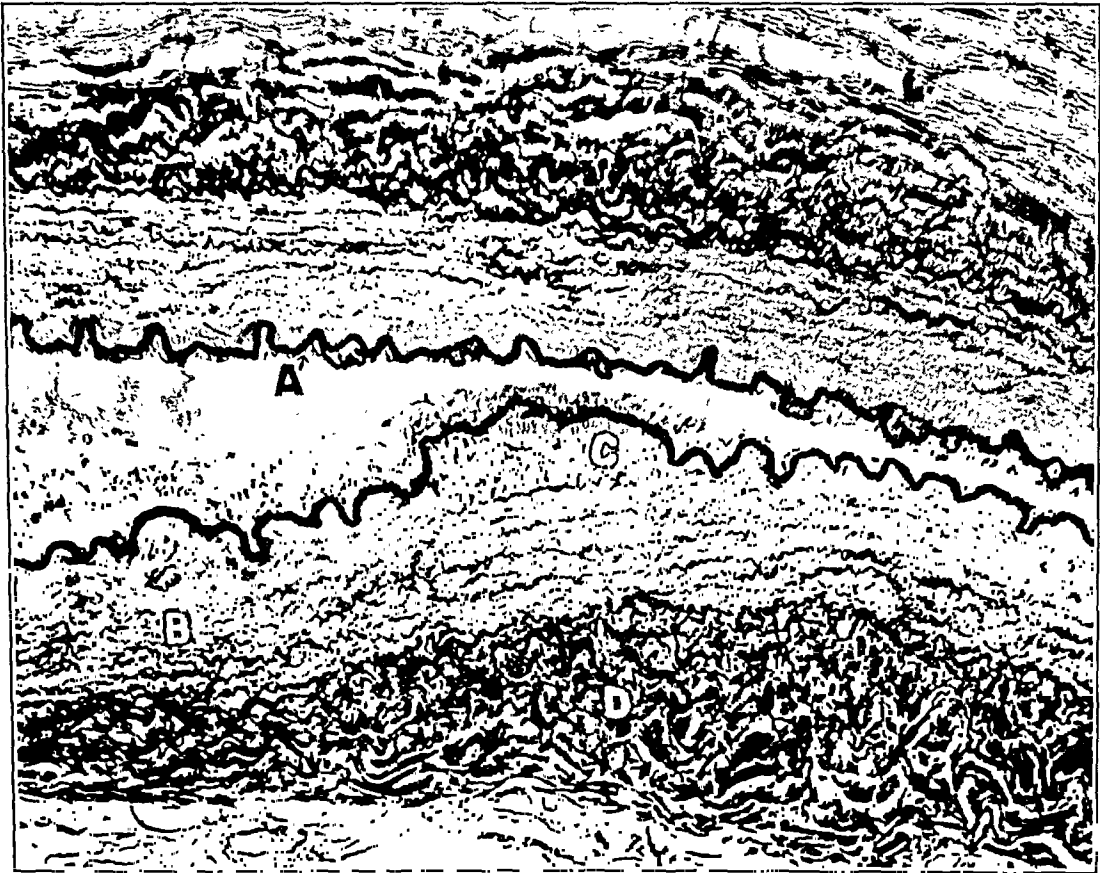
PLATE 79

FIG. 5. Left circumflex coronary artery. Age 2 months. Medium power. Cross-section. Weigert's elastic and Van Gieson's connective tissue stain.

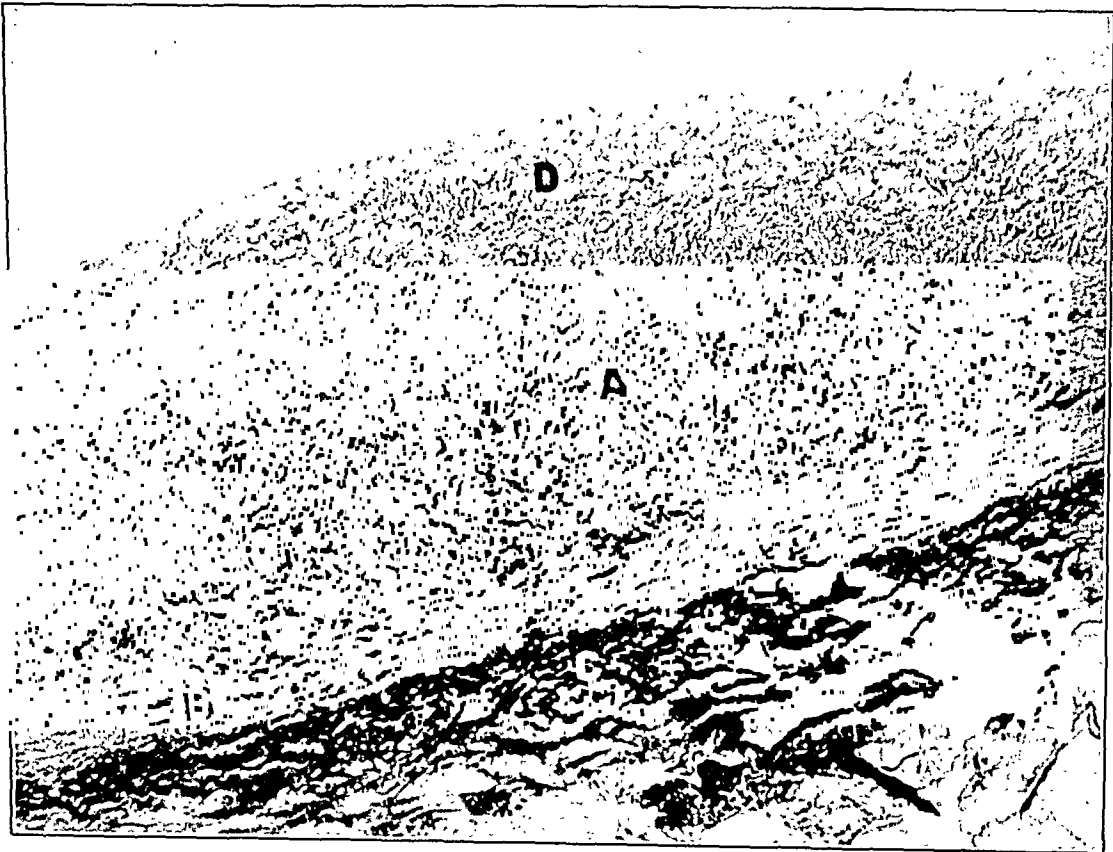
A = lamella elastica interna; B = media; C = longitudinal smooth muscle bundle in media; D = adventitia.

FIG. 6. Left circumflex coronary artery. Age 18 months. Medium power. Cross-section. Weigert's elastic and Van Gieson's connective tissue stain.

A = intermediary layer of elastic and longitudinal smooth muscle bundles. Note absence of lamella elastica interna; B = media; C = adventitia; D = intima.



5



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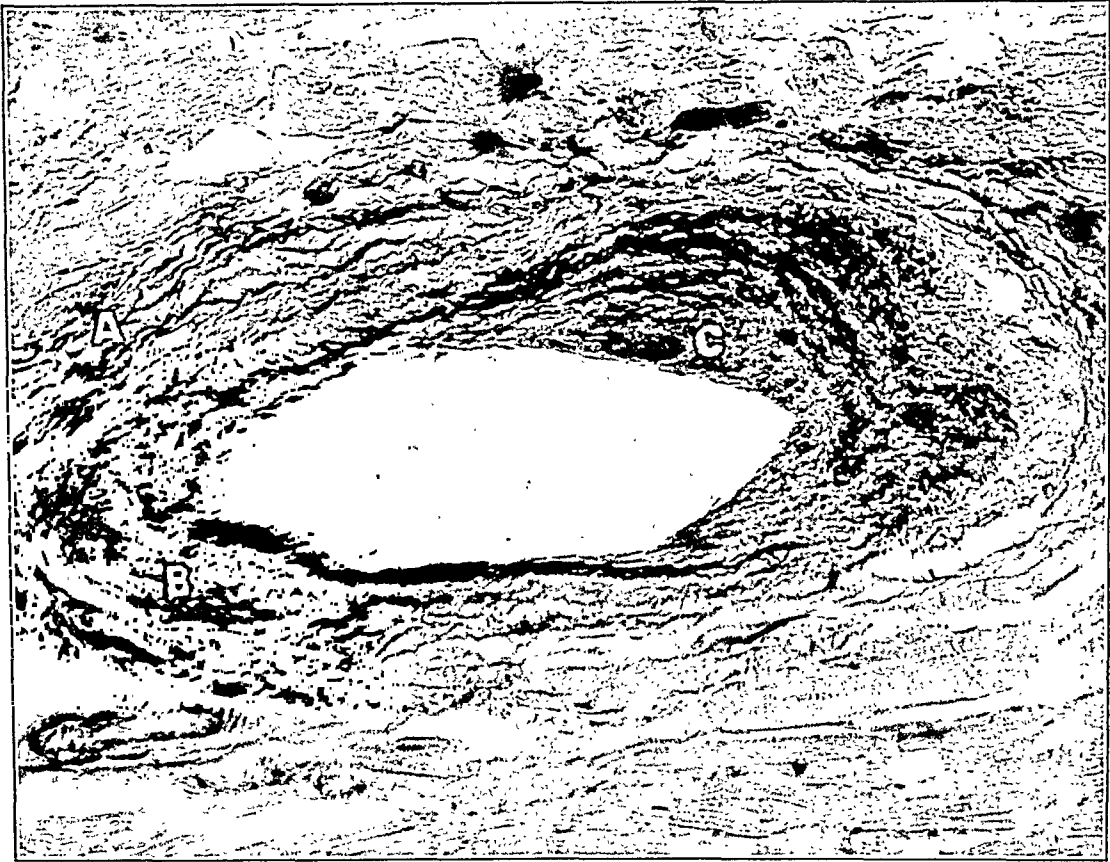
PLATE 80

FIG. 7. Myocardial artery in left posterior papillary muscle showing typical fibro-elastic transformation. Age 40 years. High power. Cross-section. Weigert's elastic and Van Gieson's connective tissue stain.

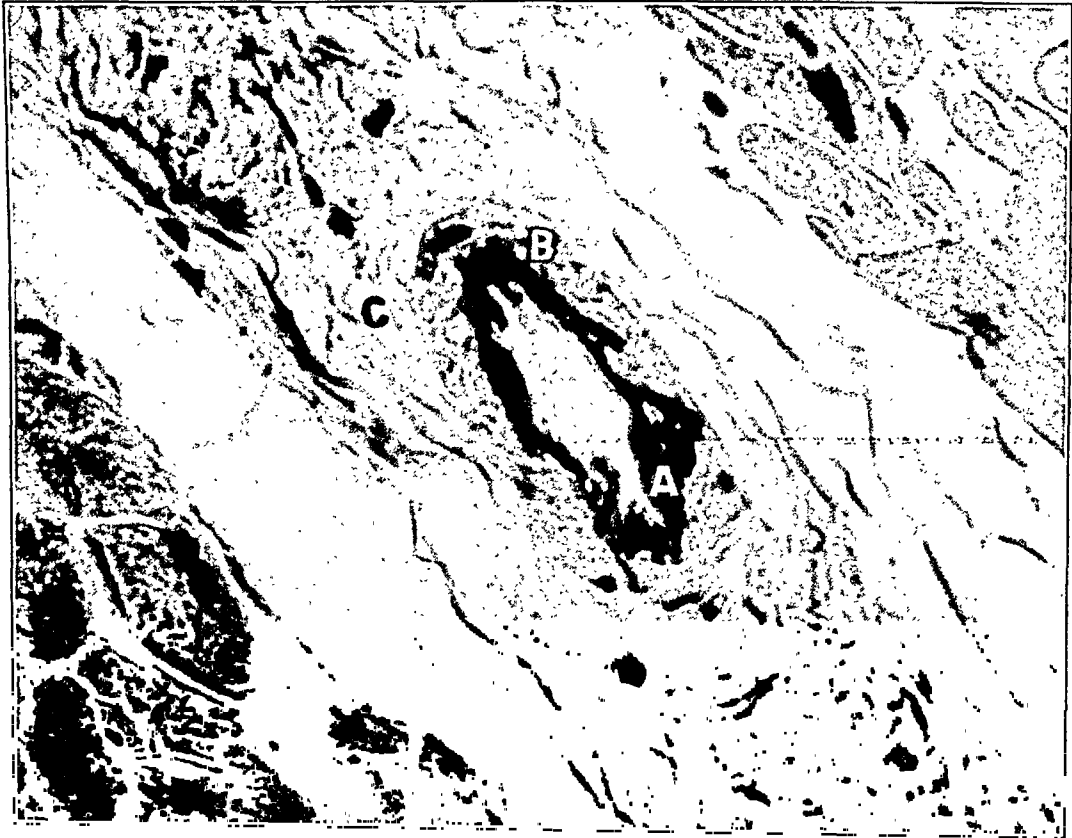
A = adventitia; B = media showing almost complete loss of smooth muscle with elastic and fibrotic changes; C = elastic-hyperplastic and fibrotic intima.

FIG. 8. Arteriole in the posterior papillary muscle of the left ventricle showing advanced intimal changes. Age 43 years. High power. Cross-section. Weigert's elastic and Van Gieson's connective tissue stain.

A = thickening and splitting of the lamella elastica interna; B = media; C = adventitia.



7



8

EXPERIMENTAL STUDIES ON VENEREAL SARCOMA OF THE DOG *

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Venereal sarcoma has been the subject of considerable experimental work following the observations of Nowinski¹ (1876) and Wehr² (1888) that it is readily transmitted from diseased to healthy dogs.

This tumor occurs as single or multiple, nodular or papillary growths commonly on the corona penis or in the vaginal mucosa. It occasionally spreads by metastasis to the nearby lymph nodes but seldom to internal organs. It is readily transmitted by sexual contact, by rubbing the tumor into scarified mucous membrane, or by injecting pieces of the tumor into the subcutaneous tissue. In most instances the tumor remains localized to the genital organs and often regresses spontaneously.

The literature on studies of venereal sarcoma has been reviewed recently by Opie³ and Feldman.⁴ The tumor has been described in Russia, the United States, France, Germany and England (Opie³), but there is no accurate information concerning its incidence. During the past 3 years 5 instances of spontaneous venereal sarcoma were observed at the University of Pennsylvania Veterinary Hospital among approximately 30,000 dogs presented for examination. This tumor was much more frequently encountered 10 to 20 years ago, according to Dr. A. Glass and Dr. W. J. Lentz of the veterinary school. It is possible that caretakers of kennels are aware of the ease with which this tumor is transmitted and destroy or segregate affected dogs without consulting a veterinarian.

The nature of this tumor is unknown. According to most investigators it is a very readily transmissible neoplasm, indistinguishable from other mammalian tumors. It was first described

* These investigations were supported by a Fund for the Study of Leukemia and Related Conditions.

Received for publication September 25, 1933.

as a carcinoma but subsequent investigators considered it a round-cell sarcoma. It is often described as a lymphosarcoma, although there is no evidence that the cells forming this tumor are lymphocytes. According to a few workers it is due to a stimulation of tissue cells of the host by a microörganism, perhaps a filterable agent, and is regarded by them as a granuloma.

GROSS AND MICROSCOPIC CHARACTERISTICS OF VENEREAL SARCOMA

Venereal sarcoma is grayish white, very firm and, unlike lymphosarcoma, cuts with difficulty and does not emulsify when cut up in Locke's solution.

Microscopically (Figs. 7 to 12) it is made up of large round cells subject to little variation in appearance. They are somewhat larger than lymphoblasts, contain a large vesicular nucleus with usually a single intensely basophilic nucleolus, and have abundant cytoplasm, slightly eosinophilic (pale in hematoxylin-eosin preparations). The cells are in contact with each other like epithelial cells, but when detached they resemble large lymphocytes. Differentiation or maturation was never seen in any direction and for this reason the nature of this tumor remains obscure. Small accumulations of lymphocytes occur in older tumors; it is apparent, however, that the tumor is invaded by the lymphocytes and that the tumor cells do not mature into small lymphocytes. Connective tissue is scant in young tumors (Fig. 7); in older tumors it sometimes becomes abundant, separating the sarcoma cells into small nests, as shown in Figure 8. Sections stained with Heidenhain's modification of Mallory's anilin blue stain (Fig. 11), and with Foot's silver stain (Fig. 12), show scant collagenous and reticulum fibers among the large round cells.

EXPERIMENTAL

Two cases of venereal sarcoma were observed by us in December 1929, and each was successfully transmitted to healthy dogs. The first occurred as a nodular growth about 1 cm. in diameter, in the vaginal mucosa of an English bulldog. Parts of the tumor were removed and injected into the groin of 2 dogs and into the peritoneal cavity of a 3rd dog, and rubbed into the scarified penis of a 4th dog. Following biopsy this apparently spontaneous tumor

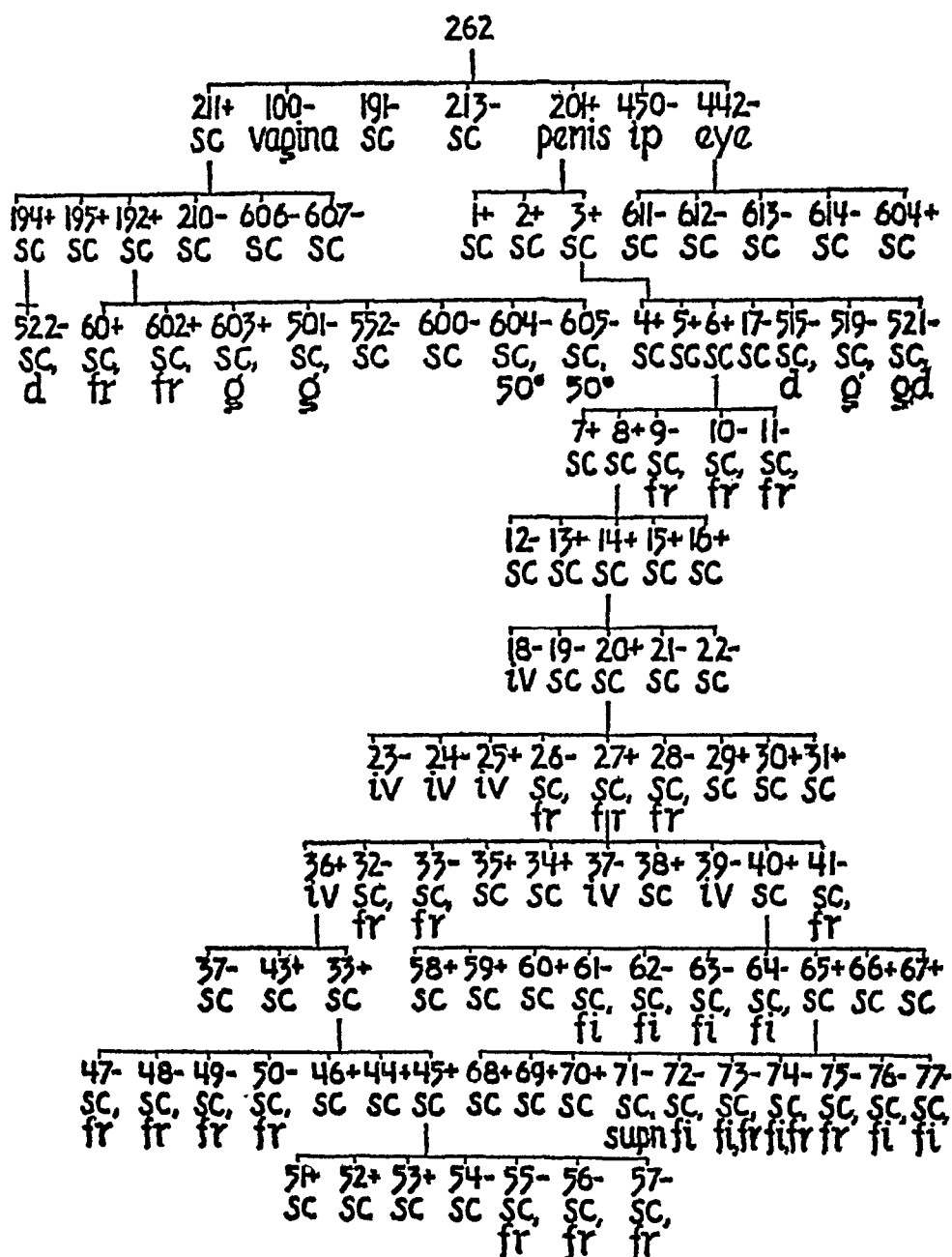


CHART 1. PASSAGES OF A TRANSMISSIBLE STRAIN OF VENEREAL SARCOMA

The following abbreviations have been used:

Route of Injections: sc = subcutaneous; ip = intraperitoneal; and iv = intravenous.

Results: + = inoculation successful; - = inoculation unsuccessful.

Material Injected: fi = filtrate; d = dried; g = glycerinated; fr = frozen and thawed tumor tissue; supn = supernatant liquid obtained by spinning emulsions of cut up tumor tissue.

disappeared. Repeated attempts to reinoculate the animal in which this tumor originated with venereal sarcoma originating in another dog were unsuccessful. Breeding of this dog was attempted 2 years later, when service was satisfactory, but conception did not occur.

The attempted transmission was successful in only 1 dog, which was injected subcutaneously. A tumor appeared at the site of injection and measured about 1 cm. in its greatest diameter, 52 days after injection. The tumor grew slowly, and within 1 month reached the size of about 6 cm. Most of it was removed, and the small part left regressed. Further transfers of this tumor were not attempted.

Transfers were more successful from a venereal tumor on the penis of a pointer (No. 262). This was a soft, gray, cauliflower-like growth approximately 3 cm. in diameter (Fig. 1), with blood oozing from its surface. Blood counts and differential counts were normal. The tumor was successfully transplanted by inoculation into 2 of 7 dogs, and during the course of 3 years 11 successive passages were made. The results of the inoculations are summarized in Chart 1.

Tumor tissue removed aseptically was cut up into small pieces in Locke's solution. Unless otherwise stated, the material injected was an emulsion of tumor cells, the route of inoculation was subcutaneous, and the site of injection the groin. In several instances simultaneous inoculations with different materials were made at different sites.

Subcutaneous Inoculations

An emulsion of tumor tissue was inoculated subcutaneously in 16 experiments and 41 of 57 dogs injected (72 per cent) developed tumors at the site of inoculation. The success of the individual experiments varied from 20 to 100 per cent. In 8 experiments all the dogs inoculated, from 2 to 4 in each, developed tumors. During the first 4 passages 8 of the 19 inoculations (42 per cent) were successful, while in the course of 10 succeeding experiments 33 of 38 (87 per cent) were successful. This increase may be due either to an increased virulence of the strain or to the fact that younger dogs were used. The exact age of these experimental animals was not known, but in the early experiments most of the dogs were comparatively old, in later experiments most of them were young.

The tumor made its appearance in from 18 to 77 days after inocu-

lation. It grew gradually until it approximated from 4 cm. to 10 cm. in the longest diameter. It regressed in most animals from 30 to 250 days after its development was first noted. The tumors were removed in many instances and in none was there any recurrence. The growth was localized to the site of inoculation. Metastasis was observed in only 1 animal. This dog, No. 40, was injected in the left groin with fresh tumor material and in the right groin with frozen and thawed material. A tumor appeared in the left groin 45 days after injection, but none in the right groin. The tumor increased gradually in size for 2 months, and later small tumors appeared in the conjunctiva of the right eye, in the wall of the right thorax, in the subcutaneous tissue in the inguinal region, and at the site of the left popliteal lymph node. Three months later a swelling occurred between the eyes, involving the frontal region. An X-ray picture showed that the skull was not affected, all the tumor being in the soft tissues outside the skull. A small piece of this tumor removed showed the characteristic appearance of venereal sarcoma, as illustrated in Figure 9.

Intravenous Inoculations

In 2 experiments intravenous inoculations were made and 2 of the 7 dogs inoculated developed fatal generalized sarcomatosis. One dog (No. 25) died after an apparent illness of about 3 weeks. Postmortem the dog was emaciated and there were many small tumors in the skin, varying in size from about 1 mm. to 1.5 cm. in diameter. There were numerous tumor masses in and beneath the mucous membrane of the mouth and conjunctiva (Fig. 3). The vagina had prominent metastatic growths measuring about 0.5 cm. in diameter. Subcutaneous tissue and muscles were studded with tumors, as shown in Fig. 2. Tumor masses were found in the intercostal muscles, lungs, diaphragm, heart, pericardium, mesentery, pancreas and kidneys, and several lymph nodes were almost completely replaced by tumor tissue, but the spleen and liver were free.

In another dog (No. 36) tumor nodules appeared 3 months after intravenous inoculation in the mucous membrane of the mouth, in the corium and in the subcutaneous tissue of skin and penis (Figs. 5 and 6). The animal gradually lost flesh and was killed 1 month after the nodules made their first appearance. The postmortem appearances were similar to those of No. 25.

Miscellaneous Inoculations with Fresh Tumor Tissue

The possibility of transmitting the disease through intact mucous membrane was tested by dropping an emulsion of tumor tissue into the conjunctiva of 3 dogs, none of which developed tumors. Tumor tissue was rubbed into the scarified mucous membrane of the penis of a dog (No. 201): a tumor appeared 70 days later and developed into a cauliflower-like growth on the glans penis. This animal is still alive 3 years after inoculation with the tumor, unchanged, as illustrated in Figure 4.

Two dogs were inoculated underneath the mucous membrane of the lip. Small tumors appeared in each. Both tumors regressed and later disappeared.

A fresh emulsion of tumor tissue was injected into the peritoneal cavity of 1 dog and similar material was rubbed into the scarified skin of another dog; neither developed tumors.

Attempts at Transmission by Material Free from Viable Cells

The experiments described below were undertaken for the purpose of determining if the agent transmitting venereal sarcoma is resistant to procedures that destroy mammalian cells but do not affect viruses.

Treatment with Glycerin: Four dogs were injected in 2 experiments with material kept in 50 per cent glycerin, for 10 days in 1 experiment and 4 months in another; none of these animals developed tumors.

Drying: Three dogs were injected with tumor tissue dried *in vacuo* under phosphoric anhydride under conditions that failed to destroy the agent transmitting leucosis⁵ of chickens, but all remained free from tumors. Thus attempts to preserve by drying the ability of the tumor tissue to produce venereal sarcoma were unsuccessful.

Filtration: Tumor tissue was cut up in Locke's solution and the centrifugalized supernatant fluid was passed through a coarse siliceous filter. Filtrate thus obtained was injected into the subcutaneous tissue of 2 dogs; the inoculations were unsuccessful. Centrifugalized supernatant unfiltered fluid produced tumors in 2 of 4 dogs injected. The cut up tumor material contained much fat and because of the possibility that fat prevented tumor cells from being thrown down by spinning, this experiment was repeated. Three dogs were injected

with supernatant fluid, but none developed tumors at the site of injection. Two of these dogs (Nos. 69 and 70) were also injected on the opposite side with uncentrifugalized tumor material and a tumor developed in each. Five dogs were injected with the supernatant fluid passed through a very coarse Berkefeld filter and all remained free of tumors. The filter used in this experiment had a bubbling pressure of 23 cm. Hg., and the flow of water during 2 minutes at 40 cm. Hg. was 200 cc.

Freezing and Thawing: Bacteria and viruses are very resistant to low temperatures but mammalian cells, on the contrary, are destroyed when exposed to temperatures below 20° C. Cramer⁶ states that sarcoma of mice contains a transmitting agent that resists freezing and thawing, but Furth, Seibold and Rathbone⁷ observed that exposure of leukemic lymphocytes to -20° C for 30 minutes abolishes the ability of these cells to transmit leukemia.

In the first 2 experiments 5 dogs were injected with tumor tissue that had been frozen in liquid air. One developed tumors at the site of injection. The test tubes containing the pieces of tumor used in these experiments, however, were not entirely submerged in the liquid air and it is possible that some of the inoculum had escaped freezing. For this reason 4 more experiments were performed in which the material was sealed in a test tube before freezing and was submerged in ether cooled to a temperature of about -70° C by the addition of large amounts of carbon dioxide ice. In these 3 experiments the venereal sarcoma lost entirely its ability to transmit the disease, for none of the 14 dogs injected developed tumors. *B. prodigiosus*, treated in a similar manner in 2 experiments, was unaffected by freezing. The unfrozen emulsion of venereal sarcoma used in these experiments produced tumors in all 13 dogs injected.

Exposure to 50° C: Sticker⁸ states that the viability of venereal sarcoma is not destroyed by exposure to a temperature of 50° C for 2 hours. Since mammalian cells are not known to survive such a temperature we have repeated this procedure, injecting 2 dogs with venereal tumor material heated to 50° C for 1½ hours; neither developed tumors.

These experiments present evidence for the assumption that venereal sarcoma cannot be transmitted by cell-free material.

Attempts at Reinoculation

Repeated attempts to reinoculate 2 dogs in which venereal sarcoma underwent spontaneous regression were unsuccessful. One of these dogs was reinjected 3 times. On the other hand, reinoculation was successful in a dog previously injected with tumor material heated to 50° C, and in 3 dogs that had been inoculated with frozen material. These observations support the assumption that the material heated to 50° C, or frozen and thawed, did not contain the agent that transmits venereal sarcoma.

Attempts of Heterotransfer of Venereal Sarcoma to Irradiated Mice

Two attempts were made to transfer venereal sarcoma of the dog to irradiated mice. In 1 experiment 9 mice that had been exposed 4 days previously to 400 r-units of X-rays were inoculated subcutaneously with an emulsion of tumor tissue. In one mouse, killed 9 days after the inoculation, no tumor tissue was recognized grossly, but microscopically (Figs. 13 and 14) tumor cells were seen in the subcutaneous tissue. The tumor cells appeared viable and a few of them were undergoing mitotic division. There was no inflammatory reaction about the area infiltrated by tumor tissue.

In another mouse, killed 11 days after inoculation, there was a tumor of about 2 mm. in size at the site of inoculation. Microscopically this tumor was seen to be surrounded by a connective tissue capsule, through which polymorphonuclear leukocytes invaded the tumor. The central part was necrotic but the cortical part contained apparently healthy tumor tissue (Fig. 15). One of the inoculated mice died 23 days after injection, the rest died or were killed from 6 to 12 days after injection, but none of them showed grossly visible tumors. In another experiment 11 mice irradiated 1 day previously were injected with venereal sarcoma, 8 subcutaneously, 3 intraperitoneally. In 3 mice injected subcutaneously the tumor reached a size of about 2 mm., in 1 about 4 mm. in diameter; the remaining subcutaneous and all the intraperitoneal injections were unsuccessful; 5 of the mice so treated were observed for a period of 2 months and showed on postmortem examination no trace of tumor tissue.

These observations show that cells of venereal sarcoma may survive and multiply for a period of approximately 9 days in the sub-

cutaneous tissue of mice in which resistance has been lowered by exposure to X-rays, but thereafter they undergo regression and disappear entirely.

DISCUSSION

The experiments described indicate that venereal sarcoma can be transmitted only by viable cells. The tumor is characterized by ease of transmission, for it can be successfully passed to dogs of any breed and, according to Sticker,⁸ to foxes as well. The natural spread by coitus was described by Smith and Washbourn,⁹ and by White.¹⁰ Our experiments show that it is the result of implantation cells, and the tumor has been incorrectly designated "infectious sarcoma of dogs."

Microscopically, venereal sarcoma possesses the characteristics of a malignant tumor; nevertheless sarcomas that are apparently spontaneous disappear or may be completely arrested by removal of most of the tumor. Their disappearance may be explained by assuming that the presumably spontaneous tumors arose by implantation of tumor cells from other diseased dogs through contact. It is noteworthy in this connection that no spontaneous neoplasm of this description was observed in the internal organs.

The origin of the cells forming this tumor is unknown. Evidence for the view that the tumor cells are lymphocytes is wanting. Indeed, they appear to have little affinity for lymphoid organs, their favorite site of growth being the subcutaneous tissue, mucous membranes and corium. The cells in this growth were never seen maturing into typical lymphocytes. For this reason its designation as lymphosarcoma has no basis. Beebe and Ewing¹¹ consider it to be either alveolar sarcoma or endothelioma. Any discussion of the cellular origin of this tumor is necessarily unsatisfactory. The tumor cells are monotonously alike; they show neither evidence of differentiation nor of organization. There is no evidence that they are reticular cells. Reticulum fibers are few if any among the tumor cells. The tumor cells, when detached, are obviously cytologically different from histiocytes. Anastomosis or formation of syncytium was not seen. Phagocytic properties were not observed, although this was not tested experimentally. Lack of formation of vascular channels or cavities by tumor cells and failure to demonstrate origin in endothelium are sufficient reason for not designating this tumor an

endothelioma, although the endothelial origin of the tumor cells is the most likely supposition, according to Ewing.¹²

This tumor may apparently arise in locations other than the genital organs. Feldman has described a tumor originating in the eye, indistinguishable microscopically from venereal sarcoma. One of the spontaneous instances of this disease observed at the veterinary school originated in the skin or subcutaneous tissue over the anterior tibial region of a male Boston terrier.

The tumor-like growth of rabbits caused by filterable viruses (myxoma of Sanarelli, *cf.* Ref.¹³) and the similar tumor described by Shope¹⁴ differ from venereal sarcoma in two respects: (1) the agents of the former tumors readily pass bacteria-tight filters and can be preserved in glycerin; and (2) they originate from the cells of the host that are stimulated to rapid reproduction by a filterable virus. Venereal sarcoma caused by transmission is the result of multiplication of the transplanted tumor cells (Beebe and Ewing¹¹) and all attempts have failed to transmit the tumor by material not containing viable cells.

SUMMARY AND CONCLUSIONS

Two venereal sarcomas have been successfully transmitted to healthy dogs, and one of them was transplanted in 11 successive generations.

Inoculations were successful in 72 per cent of the dogs injected subcutaneously with emulsion of tumor cells. Tumors appeared at the site of inoculation within an average of 38 days and with one exception began to regress after reaching a size of about 10 cm. in the longest diameter. In 1 dog the tumor spread by metastasis throughout the body.

Intravenous inoculation produced generalized sarcomatosis in 2 of 7 inoculated animals. Transmission was also successful by rubbing tumor material into the scarified surface of the glans penis. Attempts to transmit the disease through intact mucous membrane (the conjunctiva) were unsuccessful.

The ability of the tumor material to transmit the disease was destroyed by the addition of 50 per cent glycerin, by desiccation, by freezing and thawing, and by heating to 50° C for 1½ hours. Tumor material passed through siliceous filters likewise failed to produce tumors.

These experiments indicate that venereal sarcoma, often designated "infectious sarcoma of dogs," is a neoplastic process and, like other mammalian tumors, can be transmitted only by viable tumor cells.

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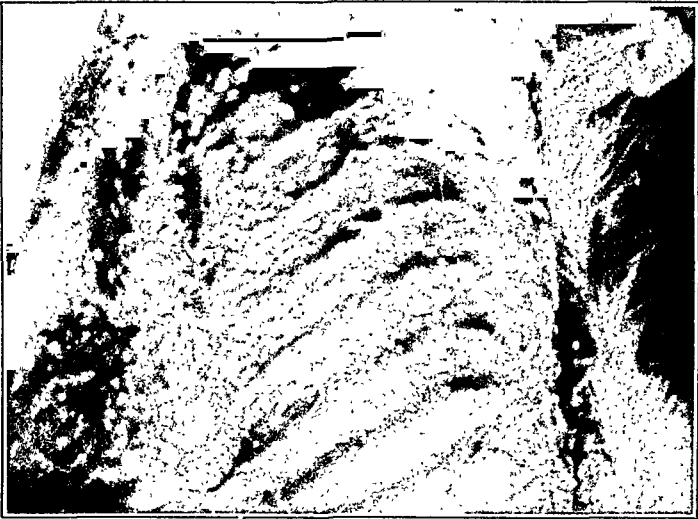
DESCRIPTION OF PLATES

PLATE 81

- FIG. 1. Spontaneous venereal sarcoma on the penis of a pointer (No. 262) in the form of a soft cauliflower-like growth of approximately 3 cm. in diameter.
- FIG. 2. Tumor nodules in the subcutaneous tissue and muscles of a dog (No. 25) caused by intravenous injection.
- FIG. 3. Tumor nodules beneath the mucous membrane of the mouth and conjunctiva caused by intravenous inoculation (No. 25).
- FIG. 4. Cauliflower-like growth of 3 years duration on the penis of a poodle, produced by rubbing tumor tissue into the scarified mucous membrane of the penis (dog No. 201).
- FIGS. 5 and 6. Generalized tumor formation in the skin and subcutaneous tissue produced by intravenous injection (dog No. 36).



1



2



3



4



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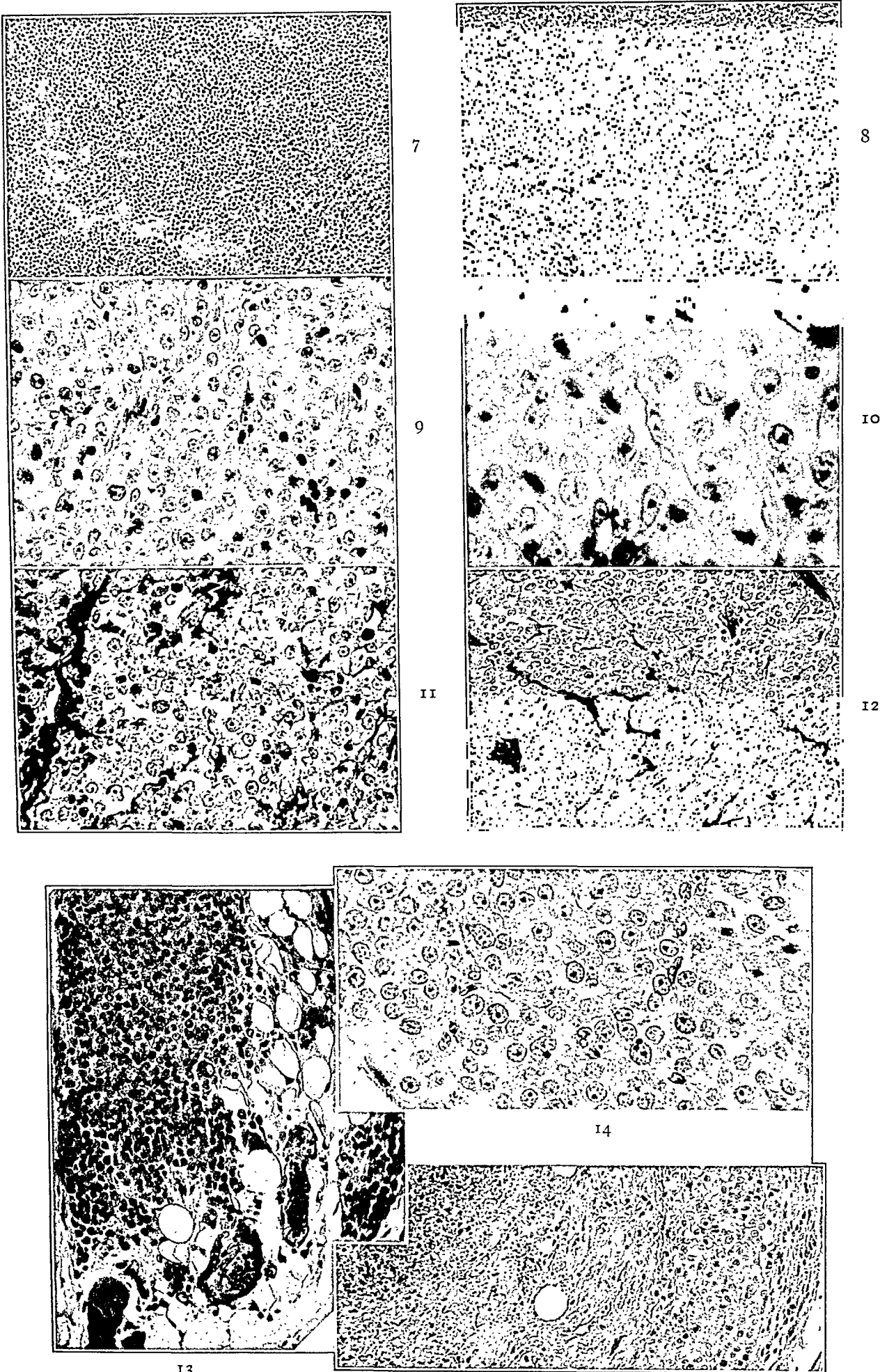
Stubbs and Furth

6

Venereal Sarcoma of the Dog

PLATE 82

- FIG. 7. Microscopic appearance of venereal sarcoma of dogs with scant amount of connective tissue. Hematoxylin and eosin. $\times 100$.
- FIG. 8. Microscopic appearance of venereal sarcoma with abundant connective tissue separating the tumor cells into small nests. Hematoxylin and eosin. $\times 100$.
- FIG. 9. Same as Fig. 7, higher magnification. $\times 400$.
- FIG. 10. Same as Figs. 7 and 9, higher magnification. $\times 900$.
- FIG. 11. Section of venereal sarcoma stained with Heidenhain's modification of Mallory's anilin blue stain, showing that fiber formation is abundant about the blood vessels, but scant if any among the tumor cells. $\times 400$.
- FIG. 12. Reticulum fibers in venereal sarcoma reproduced according to Foot's silver impregnation method. $\times 200$.
- FIG. 13. Microscopic appearance of a small tumor nodule of venereal sarcoma of dogs 0 days after subcutaneous injection in an irradiated mouse. $\times 200$.
- FIG. 14. Same as Fig. 13, higher magnification. $\times 600$.
- FIG. 15. Venereal sarcoma of dogs 11 days after subcutaneous inoculation in an irradiated mouse. The tumor is surrounded by a thick fibrous capsule. Its central part is necrotic and leukocytes invade the peripheral part, which is composed of apparently viable tumor cells. $\times 200$.





THE RENAL LESIONS OF RHEUMATIC FEVER *

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While for many years it has been recognized that rheumatic fever exerts a profound influence upon the various structures of the heart it is only recently that the possibility of the far reaching effects of this disease in other organs, and particularly in vascular tissues, has been recognized. In what manner and to what extent the kidney may be involved in rheumatic infection it has been the purpose of this study to investigate.

Although the fully developed Aschoff nodule, as seen characteristically in the myocardium, is probably the only truly specific histological expression of rheumatic fever, its presence must be regarded as representing merely one particular phase in the course of a morbid process, and as such must probably be looked upon only as an extreme manifestation of a general mode of reaction. It must be emphasized further that the rheumatic nodule cannot be regarded as a fixed or static structure, that it is undergoing continual changes, and that it probably passes through a fairly definite life cycle. That closely similar, though less specific, local inflammatory reactions may occur in relation to the smaller vessels, particularly in the kidney, we shall endeavor to point out.

Considerable attention has been devoted to this problem by various writers, the observations of Fürbringer, Baehr, Sacks, Löhlein, Fahr, Pappenheimer and VonGlahn being particularly worthy of note. Klotz, however, was the first to draw attention to the constancy with which widespread lesions occurred in the arterioles of the viscera in rheumatic fever. In the kidney he observed a non-suppurative, perivascular infiltration in relation to the smaller vessels, comparable in character to that found in the myocardium. This began in the vicinity of the intralobular arteries and spread along the course of the vessels into the cortex. Healing was observed to take place by a radiating type of fibrosis with later shrinkage and the production of a granular contracted kidney. He was able to

* Received for publication October 5, 1933.

produce similar perivascular lesions in the hearts and kidneys of rabbits by the intravenous injection of various types of streptococci.

In the present study 16 proved cases of rheumatic fever were selected, 10 of these, ranging from 18 to 52 years of age, having come to autopsy in the Toronto General Hospital, and 6 at the Hospital for Sick Children (3 of these were 3 years of age, the others 8, 10 and 13 years respectively). Definite Aschoff nodules were demonstrated in the myocardium in 13 of the 16 cases.

In the majority of instances the lesions to be described in the kidney were fairly distinctive and were present in 14 cases. These changes occurred characteristically in relation to the smaller vascular structures, particularly to the intralobular and arcuate arteries and the arterioles of the cortex. They were never observed in relation to the larger branches of the renal vessels. In general, the pathological changes were of three types. Evidence of acute or subacute inflammation was present in 8 of the 16 cases, a chronic or healed lesion was found in 4, while a recurring type of inflammatory reaction was met with twice. The various pictures may be described separately.

ACUTE NON-SUPPURATIVE PERIARTERITIS

The lesion consists essentially of a non-suppurative type of inflammation in the perivascular spaces involving the adventitia and with frequent pathological changes in the medial and intimal coats. The inflammatory exudate is made up chiefly of lymphocytes and plasma cells, together with a few indefinitely outlined cells, the nuclei of which are pale staining, vesicular and sometimes distorted in appearance. Occasional polymorphonuclear leukocytes with fragmented nuclei are also found. The stroma surrounding many of the involved vessels presents a peculiar appearance, being composed of a fine fibrillar type of connective tissue with few nuclei. In many cases the fibrils are loosely arranged and widely separated from one another, giving the appearance of a perivascular edema.

The inflammatory reaction frequently surrounds and involves only a portion of the vessel and has a somewhat nodular arrangement suggesting the focal type of reaction so often seen about the small vessels in the myocardium. In some specimens, however, the zone of inflammation surrounds the entire vessel. The inflammatory

cells lie for the most part in the periadventitial and adventitial coats, the exudate spreading apart the connective tissue fibers and sometimes replacing them. Usually the structures of the adventitia appear indefinite and have the fibrillar and edematous appearance noted above. The picture has often the appearance of a granulomatous type of reaction and has been referred to as such by Fahr. Frequently the inflammatory cells are confined to the adventitia and periadventitial structures; in some instances, however, the media is also involved. Here the same type of cells is seen lying between the muscle fibers, the normal structure being replaced by a loose fibrillar type of tissue similar to that seen in the adventitia. Many of the nuclei of the muscle cells have disappeared in that portion of the vessel most involved in the inflammatory reaction; other nuclei appear swollen or vesicular in character with loss of chromatin substance. Frequently nuclear fragments lying free in the vessel wall among the inflammatory cells are seen. The muscle cells may appear in various stages of disintegration and dissolution with considerable thinning out of the medial coat in the most involved portion of the wall. At times these changes involve the entire circumference of the vessel and occasionally complete or almost complete disintegration and necrosis of the wall has been observed.

The intimal coat of the involved vessel shows no constant or characteristic change. In the larger vessels small nodular areas of endarteritic thickening are not uncommonly met with, but are never extreme. In those vessels, however, that show marked involvement of the medial coat advancing to necrosis and disintegration of the muscle cells, an intimal change is usually noted. This consists in a marked swelling of the lining endothelial cells, which may show large rounded vesicular nuclei and pale staining cytoplasm. Sometimes in the smaller vessels this swelling is so marked that the lumen is markedly encroached upon.

Sections were stained for fibrin by the Gram-Weigert method and for elastic tissue by both Weigert's and Verhoeff's stain. A careful search was made for the lesions described by Pappenheimer and VonGlahn, namely, an exudation of fibrin beneath the endothelium and sometimes within the muscle wall, with later canalization and organization of the exudate. This lesion, which they describe as being specific, was never encountered in any of our sections. The stretching and apparent fragmentation of the internal elastic lamina

also mentioned by these observers was noted in isolated instances, but was always accompanied by an endarteritis.

The intimal lesions later described by these authors, consisting of rows or palisades of cells separated by bands of fibrin-like material and associated by these observers with rheumatic lesions in the auricular wall, were also never found. The perivascular lesions which they describe, however, have in general a resemblance to those found in the present investigation, although the latter have a more localized or nodular arrangement than the former.

HEALED PERIVASCULAR LESIONS

The exudative lesions described above represent the active phase of the inflammatory process and may justly be referred to as a nodular type of non-suppurative periarteritis. With this is associated at times a mesarteritis with partial destruction and disorganization of the muscular coats of the vessel and a variable degree of reaction in the intima. Evidence that this lesion, like all perivascular rheumatic inflammatory changes, heals with the formation of scar tissue is not lacking. Various stages in this process may be observed in the sections examined. As the acute phase of the inflammatory reaction subsides the edema surrounding the vessels disappears along with many of the inflammatory cells, fibroblasts make their appearance and the loose fibrillar stroma is gradually converted into dense scar tissue. In some of the cases studied this perivascular scarring occurs in a radiating manner in wide zones about the vessels. Frequently, irregular, wedge-shaped processes of scar tissue extend out from the vessel into the adjacent parenchyma, which to some extent is encroached upon and replaced. Not infrequently isolated glomeruli or tubular structures are seen completely surrounded by wide bands of fibrous tissue extending out from the neighboring vessels. Later, the areas of scarring become less cellular and large amounts of collagen material are laid down. With contraction of this scar tissue deformity of renal structures occurs, with finally the production of a shrunken and granular kidney. In 4 cases the organs in the gross showed a slightly thickened capsule and a markedly granular surface; in 1 of these the process was unusually well defined. In 4 others the surfaces only presented a slightly granular appearance. In none was the size of the organ considerably reduced.

In the reparative processes the adventitial coats of the affected vessels become involved in scar formation. The media, however, seems to undergo a peculiar hyaline change, and in the smaller vessels is frequently seen to be almost devoid of nuclei and to be represented by a homogeneously acellular hyaline mass, poorly differentiated from the adventitia.

RECURRENT PERIVASCULAR INFLAMMATION

In 2 of the cases studied definite evidence of a recurring process is present. Recent and acute cellular infiltration, often quite localized and focal in character, is met with in areas of old perivascular fibrosis in which considerable collagen material has been deposited. Evidence of an accompanying edema is not marked but frequently areas in which necrosis of collagen fibrils has occurred in relation to the inflammatory process are noted.

Several times evidence of what appears to be a purely degenerative change, not directly associated with any of the above lesions, is present in many of the small arteries and arterioles. This is particularly well seen in 2 cases in which the walls of many of the finer vascular structures are considerably thickened and completely or almost completely replaced by pink-staining, granular, acellular material. The muscle fibers of the media have lost their identity and the nuclei have disappeared. The picture suggests necrosis of the vessel wall, associated with the inflammatory lesions described above. In some instances a hyaline-like change within the wall is noted. Moreover, considerable thickening of the afferent glomerular arteriole also is sometimes present. Frequently, but not invariably, associated with this is a well marked change in the corresponding glomerulus, the latter showing an atrophy, collapse and hyalinization of the capillary loops and occasionally of the entire glomerular tuft. This picture has been noted by many observers, occurring both in rheumatic and in non-rheumatic conditions. Its significance, however, is little understood, some claiming it to be purely secondary and dependent upon glomerular changes, others being of the opinion that the lesion is of primary importance in bringing about atrophy of the capillary loops through nutritional disturbances. Fahr has noted an inflammatory infiltration about this region of the afferent arteriole and describes it as being of a

granulomatous character, leading to closure of the lumen and necrosis with later hyalinization of the wall. This, although aptly describing the changes observed in many of the smaller vessels and arterioles in the cases under discussion, was not observed at the point of entrance to the afferent vessel.

GLOMERULAR CHANGES

As pointed out by many observers, the occurrence of acute glomerulonephritis in association with rheumatic fever is rare. In the present series this was never observed. Glomerular damage is present only occasionally. Only once was evidence found to suggest an inflammatory lesion of the glomeruli. This appears in a child (C-152-32) of 8 years, dying in about the third week of illness, with evidence of a subacute inflammation in occasional glomeruli. In none of the others is any endothelial or epithelial proliferation of the glomerular structures noted. Inflammatory infiltration and capsule adhesions are entirely absent. In only a few, where a glomerulus lies in close proximity to a focus of inflammation about a vessel, is there any fibrous thickening of Bowman's capsule or any swelling of the lining endothelial cells. The glomerular damage when present consists of hyalinized structures, occurring singly or at times in groups. Those glomeruli lying directly beneath the capsule are most frequently affected and small roughly wedge-shaped areas of scarring containing one or more hyalinized glomeruli are occasionally seen, representing areas of early arteriosclerotic atrophy. The involved glomeruli throughout the section are seen to be undergoing a bland type of hyalinization without, as mentioned above, showing evidence of a previous or co-existing inflammatory change. This observation lends strong support to the view that the glomerular damage present in these cases is not primarily of inflammatory origin, but rather secondary to and dependent upon nutritional disturbance brought about by vascular change.

TUBULAR CHANGES

Relatively little alteration is noted in the tubular structures apart from occasional atrophy and disappearance of those associated with obliterated glomeruli. At times scar tissue extending outward from a vessel is seen to surround and distort adjacent

tubular structures. A moderate nephrosis is present in 1 case. Sometimes granular debris and occasional hyaline casts are noted in the tubules. Well marked passive congestion is often present.

ADDITIONAL CASES REVIEWED

In addition to the above study all cases presenting evidence of rheumatic heart disease coming to autopsy during the last seven years were reviewed. Out of 2400 autopsies, 128 (5.3 per cent) were found showing either healed or active cardiac lesions. Of this group interstitial nephritis had been diagnosed in the gross in 38 (30 per cent). Half of these had occurred in individuals under 40 years of age and one-third of them in those under 30 years of age. These figures serve to illustrate two points: first, that a very definite association exists between rheumatic fever and renal disease, and second, that a high percentage of these individuals show evidence of chronic kidney damage at a very early period in life. Arteriosclerotic atrophy of the kidney, on the other hand, which was met with in 19 of the 128 cases (15 per cent) occurred in the great majority beyond the age of 50. This is in keeping with the observation that only the smaller arteries and arterioles are involved in the pathological process under discussion.

DISCUSSION

From a review of the literature it is apparent that involvement of the vascular system is a common accompaniment, if not indeed a constant manifestation, of rheumatic infection. While it has been shown that a very close relation exists between lesions appearing in the heart and arterial system, the degree of pathological alteration observed in any particular organ is subject to wide variation. It is clear, however, that the lesions induced by a rheumatic infection have certain well defined and characteristic manifestations in whatever tissue or organ they may be found, and that a common mode of reaction is always present. The most common and characteristic expression of rheumatic infection is in a non-suppurative perivascular reaction affecting chiefly the smaller vessels, associated with edema and round cell infiltration, and leading to the formation of new connective tissue. We are becoming more and more convinced that the pathological changes taking place in this condition are largely dependent upon a primary vascular lesion.

It would appear that this reaction and its influence upon the kidney structure constitutes a definite type of interstitial nephritis. While the balance of evidence would suggest that the glomerular change is a result of a nutritional disturbance and not primarily of inflammatory origin, it is easily conceivable that the same injurious agent that is responsible for the vascular lesion might occasionally lead to direct glomerular damage. As Klotz long ago pointed out, vascular changes in the kidney do not occur apart from inflammation, and it is to a common type of injury that both vascular structures and parenchymal tissue react.

While we believe that the changes noted are of frequent occurrence and give rise to a definite type of interstitial nephritis, the renal damage is only occasionally of sufficient severity to attract the attention of the clinician and lead to a diagnosis of kidney disease during life. In the acute stages of rheumatic fever small traces of albumin in the urine, which are frequently encountered, are usually ascribed to other causes. On the other hand a failing heart, with an associated congestion of the kidneys, very materially obscures the picture of an underlying nephritis toward the terminal stages of the disease. It must be observed, however, that in recurring infections the repeated injury, which plays such an important rôle in the pathological changes within the heart, may in a similar manner be suffered by the kidney, leading to a gradually diminishing reserve in the functioning power of that organ.

We cannot, however, assert that the changes which we have described in association with rheumatic fever may not be clearly reproduced by other forms of infection, particularly those of streptococcic origin. None of the lesions described as specific manifestations in the kidney by certain authors, particularly Pappenheimer and VonGlahn, has in our experience been observed. It would seem, however, that a mode of reaction in general similar in character and comparable to that found in the myocardium and wall of the aorta may take place in many of the peripheral vessels. Until more light has been thrown upon the etiology of rheumatic fever the part played by this disease in nephritis is open to debate. That such a relation exists is, we believe, beyond question. It must be emphasized that the changes observed have an irregular distribution, and that, as a rule, only relatively few vessels are involved in the pathological processes described. Thus, in general, insufficient

damage is brought about to impair renal function seriously, and it is only exceptional that evidence of kidney disease is discovered during life. It must be stated definitely, however, that in a large percentage of cases of rheumatic fever the kidneys, as well probably as many other organs, do not entirely escape, and will exhibit evidence of acute, recurring or chronic vascular damage, reflecting itself in a varying degree of pathological alteration of renal structure.

SUMMARY AND CONCLUSIONS

1. A study of the kidney lesions in 16 cases of rheumatic fever was undertaken.
2. A perivascular inflammatory reaction of the acute non-suppurative type, affecting the smaller arteries and arterioles was present in 8 cases. Evidence of perivascular scarring was noted in 4 cases, while a recurrent type of inflammation was met with in 2.
3. The inflammatory reaction is usually seen in the adventitia and periadventitial tissues, with occasional infiltration and destructive change in the medial coat. Intimal changes, consisting of an endothelial swelling and proliferation, are inconstant.
4. Glomerular damage, which was only well marked in 1 case, is to be regarded as dependent chiefly upon nutritional disturbances brought about by vascular changes. Little evidence of active or healed inflammatory processes was met with in the glomeruli.
5. No evidence of the specific vascular lesions described by Pappenheimer and VonGlahn was met with in the cases studied.
6. The lesions described, which in general bear a close resemblance to perivascular foci of inflammation found in the myocardium, may be looked upon as constituting a definite type of interstitial nephritis. It is seldom, however, that sufficient alteration in structure to justify a diagnosis of renal disease during life occurs.

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PRIMARY AMYLOID DISEASE OF THE HEART *

REPORT OF A CASE

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Although primary amyloid disease of the heart is very uncommon, the cases that have been reported in the literature¹⁻⁴ indicate that the hyaline substance may be deposited in the epicardium, myocardium, endocardium, valves, or in the walls of adjacent blood vessels. Pronounced involvement of the endocardium, the superior and inferior venae cavae and the pulmonary artery was found in a case seen recently. Since the literature on this subject does not include illustrations of lesions having the distribution indicated it is the purpose of the present communication to record pictorially amyloid infiltration of the vascular lining of the heart and blood vessels. Also, Mayer's method of staining amyloid in paraffin sections⁵ is described because it has been found worthy of more general application.

REPORT OF CASE

Clinical History: A 75 year old white male was admitted to St. Vincent's Hospital, Los Angeles, on July 13, 1932, with the chief complaint of hematuria. He had suffered from nocturia three times nightly for some time and there had been difficulty in starting the stream. Urination was not painful, although it was slow and the stream was intermittent. On June 30, 1932, dark red clots of blood appeared in the urine, which cleared up after four to five urinations and remained clear until the night before admission when bloody urine was again noticed. There was never any pain although there had been soreness above the symphysis. The patient had had typhoid at 18 years of age, malaria at 20, "kidney colic" at 40 and inflammation of the gall-bladder 1 year ago. There were no cardiac complaints.

Physical examination revealed a well preserved, elderly male who weighed 210 pounds. The blood pressure was 130/72 and the pulse 80. Examination of the head, neck, chest and abdomen gave negative results. Upon rectal examination the right lobe of the prostate was 4 plus enlarged, firm and nodular, while the left lobe was smaller in size and smooth over the surface. The bladder contained 75 cc. of residual urine. Cystoscopic examination of the bladder revealed old and recent blood clots that obscured the base, but the lateral walls and dome were normal.

* Received for publication September 11, 1933.

The urine was free of sugar but showed a trace of albumin with a few erythrocytes and pus cells. The hemoglobin was 70 per cent (Sahli) and the white blood cells numbered 10,800 with 73 per cent polymorphonuclear leukocytes. The blood urea was 34 mg. Roentgenological examination of the chest showed the heart shadow moderately enlarged but the lung fields were normal. Films of the kidneys, ureters, bladder and pelvic bones were negative for calcareous deposits and metastases.

The diagnosis of carcinoma of the prostate gland was at first considered, but following cystoscopy it was thought that the bleeding was due to an enlarged, benign, intravesical prostate. Suprapubic drainage of the bladder was advised and was carried out. A tumor 3 cm. in diameter was found situated in the base of the bladder anterior to the right ureteral orifice, with two secondary nodules about 1 cm. in diameter to the right of the internal urethral orifice. A segmental resection of the bladder, including the tumor, was made and the bladder reconstructed. The lesion was reported as carcinoma of the bladder.

The patient reacted satisfactorily to the operation and the postoperative course was essentially uneventful. The blood pressure remained about 140/60. The pulse remained full, although there was some irregularity with occasional extra systoles. He was discharged from the hospital July 19, 1932, wearing a urethral catheter, and there was still some drainage from the suprapubic wound. He was readmitted to the hospital Nov. 7, 1932, nearly 5 months after operation, because of suprapubic extravasation of urine and inability to get along without the urethral catheter. Examination of the heart at this time revealed a blowing systolic murmur over the aortic area, which was also heard over the apex and was transmitted to the axilla. Suprapubic drainage was again instituted and extensive recurring carcinoma was found in the bladder. He returned home Nov. 18, 1932, where he remained until his death, May 8, 1933. During this interval hard tumor masses appeared in the abdomen, which attained a very large size. There were symptoms and findings suggestive of bilateral ureteral obstruction with marked urinary sepsis.

An autopsy was performed 12 hours after death. Since the lesions that constitute the subject of this report were found solely in the heart and great vessels, only these structures are described in detail.

GROSS PATHOLOGY OF HEART

The heart weighed 500 gm. and was symmetrically enlarged. The serosal surfaces were smooth and glistening and a considerable amount of subepicardial adipose tissue largely obscured the musculature. This extended well upward over the pulmonary artery and systemic aorta. Upon exposing the endocardium of the right auricle the surface was covered by numerous closely placed translucent nodules that were scarcely more than pin-point in size. Upon touching them lightly with the finger tips they were easily palpated and imparted the sensation of a finely sanded surface. The identity of this process was not appreciated until after microscopic sections

were studied, when it became apparent that the glassy material was amyloid. Its distribution could be more accurately estimated after staining the heart with iodine (Fig. 1). Nodules extended over the surfaces of the superior and inferior venae cavae for a distance, but there seemed to be a rather sharp line of demarcation in the superior vena cava, beyond which the vessel was normal. Amyloid was also quite conspicuous over the valve of the coronary sinus, as well as in a Chiari network,⁶ which coursed along the superior surface of the auricle from the left margin of the superior vena cava. The foramen ovale was widely patent and the thin membrane that guarded the opening was covered by conglomerate foci. The leaflets of the tricuspid valve and even the chordae tendineae were surprisingly free of amyloid. Nodules were quite numerous in the endocardium of the right ventricle, however, (Fig. 2), being most conspicuous just below the pulmonary valve. The leaflets of the valve were quite free of disease, except close to the line of attachment where occasional stained areas were present. From the valve almost to the bifurcation the intima of the pulmonary artery was peppered with amyloid, but distally a normal structure was assumed. The endocardium of the left auricle (Fig. 3) showed smaller and probably fewer foci of hyaline material than the right auricle, but maximum involvement in both was present in the membranous interauricular septum. Practically no amyloid was found in the mitral valve leaflets, chordae tendineae, or on the surface of the papillary muscles, although several glistening, translucent vegetations 2 to 3 mm. in diameter were present along the free edge at the left angle of the valve and along the midportion of the anterior leaflet. Nodules of amyloid were less numerous in the endocardium of the left ventricle. Upon exposing the aortic valve there were a considerable number of rounded, calcified masses 2 to 4 mm. in diameter present over both surfaces of all the leaflets, and calcified adhesions were found between the free edges of the leaflets at the right anterior commissure for 0.8 cm. from the aorta. There was no calcification in the aortic wall of the sinuses and the intima of the aorta showed only occasional streaks and elevated plaques of yellowish, opaque, atheromatous material. No amyloid was seen in either the valve or the artery. The coronary orifices were unobstructed and when the vessels were followed by serial cross-sections the walls showed only moderate atheromatous changes. No amyloid was found in the intima or media of the major

arteries, although the walls of the coronary veins were heavily infiltrated. The myocardium throughout was somewhat pale but moderately firm and uniform in consistence and texture. There was but the slightest suggestion of a glassy mottling in the unstained tissue, but a section through the midportion of the left lateral wall of the left ventricle, when stained with iodine, showed a considerable amount of amyloid in the myocardium (Fig. 4). This was also true of the right ventricle and the walls of both auricles.

MICROSCOPIC EXAMINATION

A small piece of endocardium from the left auricle was stained in iodine and examined with the low power objective, using reflected light (Fig. 5). The surface showed a mosaic pattern, being divided into oblong, rhomboid and polygonal areas less than 1 mm. in diameter by sharp sulci, which intersected at all angles. Occasionally the grooves were parallel and formed longitudinal folds. The surface amyloid was largely limited to the summits of such areas and although it extended down over the margins for a distance in the zones of heavier deposit, it was uncommonly found in the troughs. In its finest form it was deposited as rounded, sharply circumscribed nodules 50 to 100 microns in diameter. Where these were closely placed they were likely to coalesce and the fused nodules formed bizarre patterns of various sizes and shapes which might be triangular, rod-like or quite rounded. Where coalescence had resulted in larger accumulations the amyloid was distributed in some variation of star-shape. Some were quite perfect six-pointed stars that presented a stippled appearance; others showed a central nucleus about which there were bar-like striae radially placed. The summit of a longitudinal fold, which was covered by many closely packed star-shaped masses of amyloid, was not unlike the appearance of a chain of mountains on a relief map. The various figures and patterns that could be found were limited only by the imagination of the observer. Myocardium from the left auricle, which was cut parallel to the surface, was similarly studied (Fig. 6). There were bundles of fine, white, opaque fibrils that ran in many directions and formed a tightly woven meshwork, the interstices of which contained vacuolated tissue. This meshwork of fibrils showed a tigroid mottling of alternating dark brown and pale yellow zones, which were short, broad and feather-edged. Closer inspection showed the

deeply staining bands crossed by fine white parallel fibers, which varied in caliber from one place to another and which anastomosed and branched. The paler areas were striated by fine brown streaks that often connected one dark zone with another. It was obvious that the pale fibrils were muscle cells and the dark material was amyloid. In the dark zones thin muscle fibers were seen against a dark background of stained amyloid, and in the light areas narrow streaks of amyloid were seen against a pale background of muscle tissue. The amount of amyloid varied from place to place and alternating zones of about equal size contained large and small quantities. A fine white stippling over a dark brown field was noted in the rare areas in which a muscle bundle was seen in cross-section. In the adipose tissue the individual fat globules were brought into sharp relief by amyloid that was deposited between cells.

Tissue for paraffin sections was taken from many areas of the auricles, ventricles, valves and large blood vessels, some of the typical lesions of which will be described.

Pulmonary Artery: Rounded, ovoid and lenticular areas of hyaline material are found in the intima, media and adventitia (Fig. 7). They are quite dense, homogeneous, acellular and sharply demarcated as a rule, being more numerous superficially, with many bulging toward the lumen from just beneath the internal elastic membrane. Although elastic fibers can be traced coursing through an area they usually end rather abruptly at the margin. The internal elastic membrane sometimes splits to surround a nodule.

Endocardium: In the endocardium of the auricles amyloid is deposited in much more irregular plaques which vary greatly in size and have a tendency to spread along the surface and coalesce (Figs. 8, 9 and 10). Many plaques which extend to the surface are covered only by endothelium. Some present a palisade effect and are the full thickness of the endocardium, while others are only in the deeper layers.

Myocardium: The amyloid has a very patchy distribution. It can easily be identified appearing in the fibrous stroma and frequently is closely applied to the individual muscle fiber, forming a sheath or tube of narrow or broad dimension which completely encircles the cell. In places it is seen only in the walls of blood vessels and the stroma itself is free. The muscle fibers in many instances appear of normal size and their finer structures are well

preserved. Atrophy is noted, however, in foci of more dense accumulation with some fibers entirely missing, leaving unstained vacuoles in a mass of amyloid. Involvement of the musculature near the epicardium seems no different from that of the central part or near the endocardium, although it is most extensive in the wall of the right auricle with the left auricle and the right and left ventricles next in order.

Pericardium: Amyloid is found in two locations, chiefly close to the myocardium, where it has been deposited between the fat cells to a moderate degree. It is also seen in the walls of smaller vessels, especially veins. Plaques of amyloid beneath the serous surface are not found.

The other postmortem findings were largely those of extensive neoplastic involvement of the prostate, bladder, suprapubic sinus tract, retroperitoneal tissues, lymph nodes, kidneys, pancreas and mesentery. There was obstruction of both ureters with marked hydronephrosis on the left and pyonephrosis on the right. No metastases were demonstrated in the liver and lung, or in the vertebrae and pelvic bones exposed. The tumor microscopically is an adenocarcinoma, being primary in the prostate. There is no microscopic evidence of amyloid in the lung, spleen, liver, pancreas, colon, adrenal, kidney, bladder, prostate, lymph node or tumor tissue. An examination of the nervous system was not made.

Pathological Diagnoses: Extensive carcinoma of prostate with extension to the bladder, suprapubic sinus tract, retroperitoneal, periaortic and mesenteric lymph nodes, kidneys and pancreas; amyloid disease of the heart, pulmonary artery and venae cavae; endocarditis, old with aortic stenosis; endocarditis, acute (mitral); hydronephrosis with pyonephrosis (right); acute ulcerations of stomach; old cholecystitis with mucocele; multiple infarctions of spleen.

METHODS

The stains employed to identify amyloid were iodine and gentian violet. For gross staining the whole heart was placed in a 5 per cent alcoholic solution of iodine and when well stained was washed in water to which a few drops of sulphuric acid were added. About 3 days were required for complete destaining and photographs were

taken when the desired degree of contrast appeared, which was after 24 hours in this instance. In photographing the specimen a strong yellow filter such as the Wratten "G" helped the contrast, amyloid appearing black in the picture.

Paraffin sections were stained with gentian violet after Mayer's method, as described by Mallory and Parker.⁵ Formalin-fixed tissues were embedded in paraffin and cut into suitable ribbons. One, two or three slices were cut from the ribbon with a scalpel and allowed to spread by transferring with a camel's hair brush to a small dish of distilled water which had been heated to 108° F. When completely spread they were pulled upon a glass slide and transferred to a warmed 0.5 per cent aqueous solution of gentian violet and allowed to stain 2 to 5 minutes. Staining was controlled with the microscope and as soon as the amyloid had a good pink color, with the remaining tissue purple, the sections were quickly washed in warmed distilled water and allowed to differentiate in $\frac{1}{4}$ to $\frac{1}{2}$ per cent acetic acid. Differentiation was also controlled microscopically and as soon as completed, usually in 30 to 60 seconds, the sections were quickly washed in fresh warm distilled water and mounted on clean glass slides without using glycerin-albumin. Drying over night at room temperature was usually sufficient, although it was sometimes necessary to warm in the 37° C incubator. After thorough drying the sections were dipped in fresh xylol 1 to 2 minutes to deparaffinate and clear and were mounted in gum damar.

The procedures can be very conveniently carried out by employing small oblong staining dishes of about 100 cc. capacity in which the solutions are kept at the proper temperature on a hot plate. In the absence of a constant temperature hot plate the jars were placed in a 12 inch pan filled with water which was kept at 105° F by a small alcohol lamp. It was easy to transfer the paraffinated sections from solution to solution by pulling them onto a glass slide with a dissecting needle or camel's hair brush.

When the sections were placed directly in the stain, without first allowing them to spread, the stain was likely to be uneven. Overstaining destroyed the contrast between amyloid and fibrous tissue and overtreatment with acid resulted in very pale, poorly contrasting sections. Moisture interfered with the preservation of the stain and could easily be detected before deparaffinating by examining the section with the low power objective, using diminished illumi-

nation. A strong green filter such as a Wratten "B" was most useful in photographing the gentian violet stain, amyloid appearing black in the picture.

DISCUSSION

Cases of amyloid disease of the heart fall into one of three general classes, depending upon the deposition of the hyaline substance in other tissues and organs of the body. In the largest group of cases of generalized amyloidosis, diffuse infiltrations affect various parenchymatous organs, such as the liver, spleen, kidney, adrenal, pancreas and, not infrequently, the heart. In generalized amyloidosis of muscular systems,^{7,8} there is an atypical distribution of amyloid which affects exclusively the cardiac, skeletal and smooth muscle tissues of the body. Those cases in which amyloid infiltration is confined primarily to the heart constitute the final group.

Since the condition in the present case was not appreciated at the time of postmortem examination extensive investigation of the skeletal muscles of the head, extremities and trunk was not made, although the smooth muscle of the bladder and gastro-intestinal tract was examined both grossly and microscopically. The absence of clinical evidence of skeletal muscle disease, and the finding of amyloid only in the locations described, would seem to warrant the conclusion that this case is one of primary amyloidosis of the heart. The amount of amyloid present in the myocardium is, perhaps, less than has been described in previous cases and, so far as is known, a partial heart block was the only specific clinical observation that might have been caused by the disease. The great importance of the heart lesion as a cause of death is questionable, but it undoubtedly contributed to the final cardiac failure.

SUMMARY

1. A case of primary amyloid disease of the heart is reported both descriptively and pictorially.
2. Mayer's method for staining amyloid in paraffin sections is described.

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DESCRIPTION OF PLATES

PLATE 83

FIG. 1. Right auricle. Note amyloid in the auricular endocardium, superior vena cava, valve of the coronary sinus, and the Chiari network. Involvement of the tricuspid valve is seen only near the margin of attachment. Iodine stain; about $\frac{3}{4}$ natural size.

FIG. 2. Right ventricle, conus arteriosus, pulmonary valve and pulmonary artery. Iodine stain; $\frac{5}{8}$ natural size.

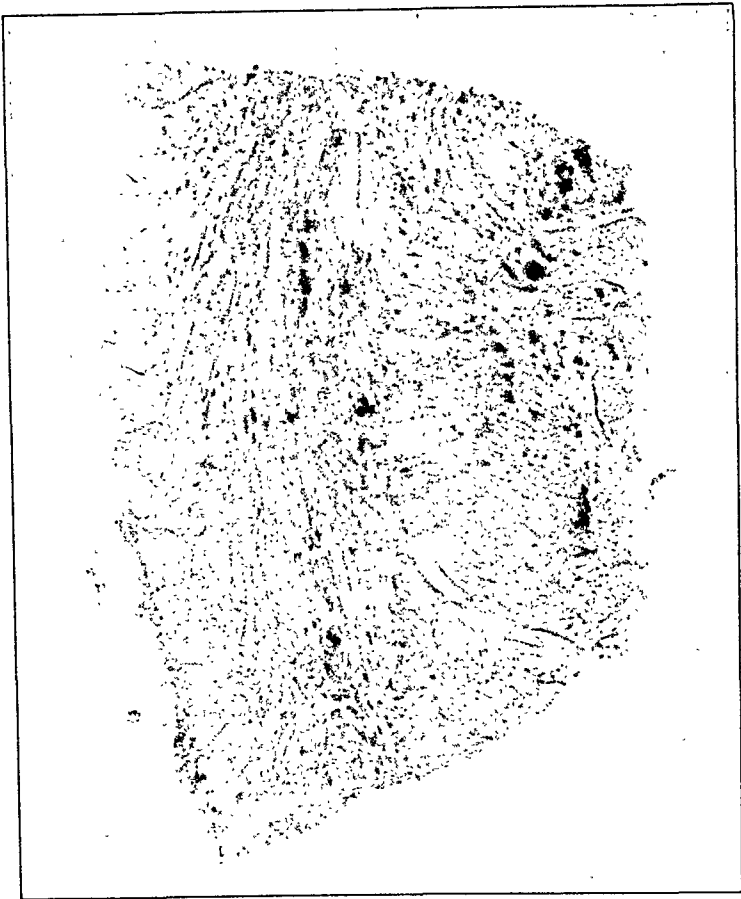


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PLATE 84

FIG. 3. Left auricle, mitral valve and left ventricle. Iodine stain; $\frac{5}{6}$ natural size.

FIG. 4. Cross-section of left ventricle. Iodine stain. $\times 3.5$.



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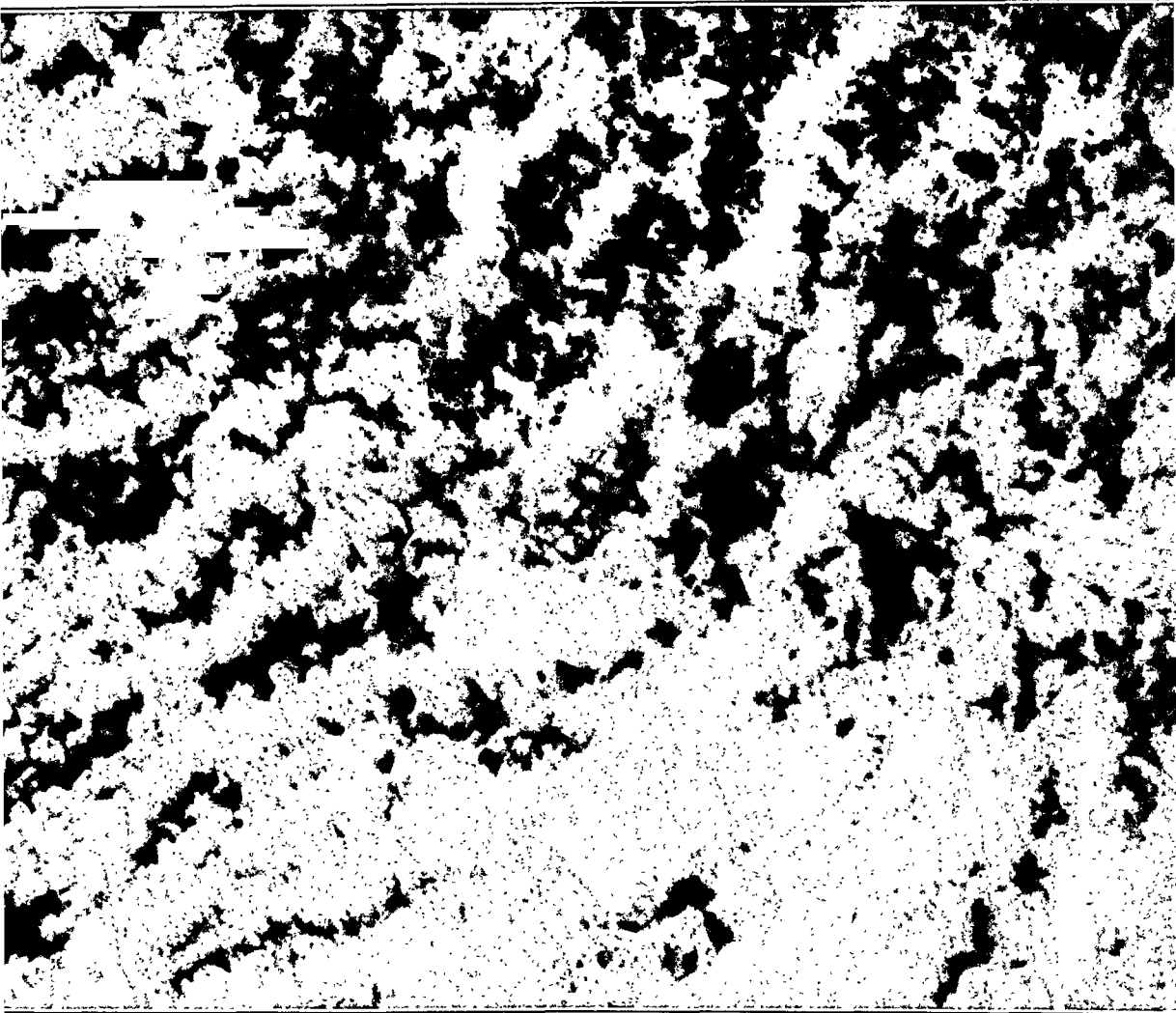


PLATE 85

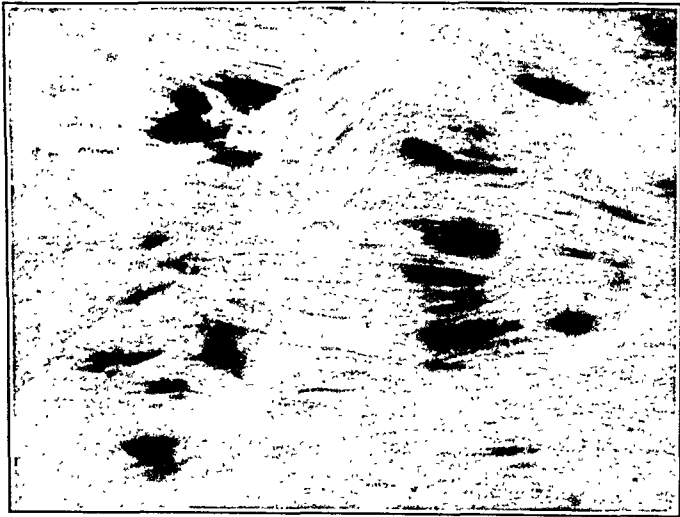
FIG. 5. Surface of endocardium of right auricle. Iodine stain. $\times 13$.

FIG. 6. Cut surface of myocardium of right auricle. Iodine stain. $\times 18$.

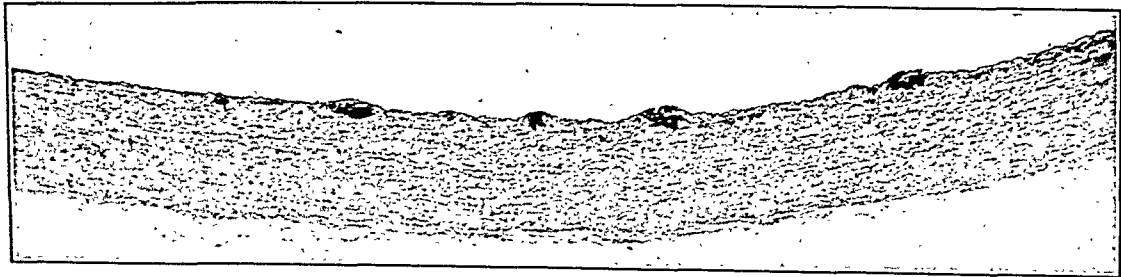
FIG. 7. Cross-section of pulmonary artery. Gentian violet stain. $\times 25$.



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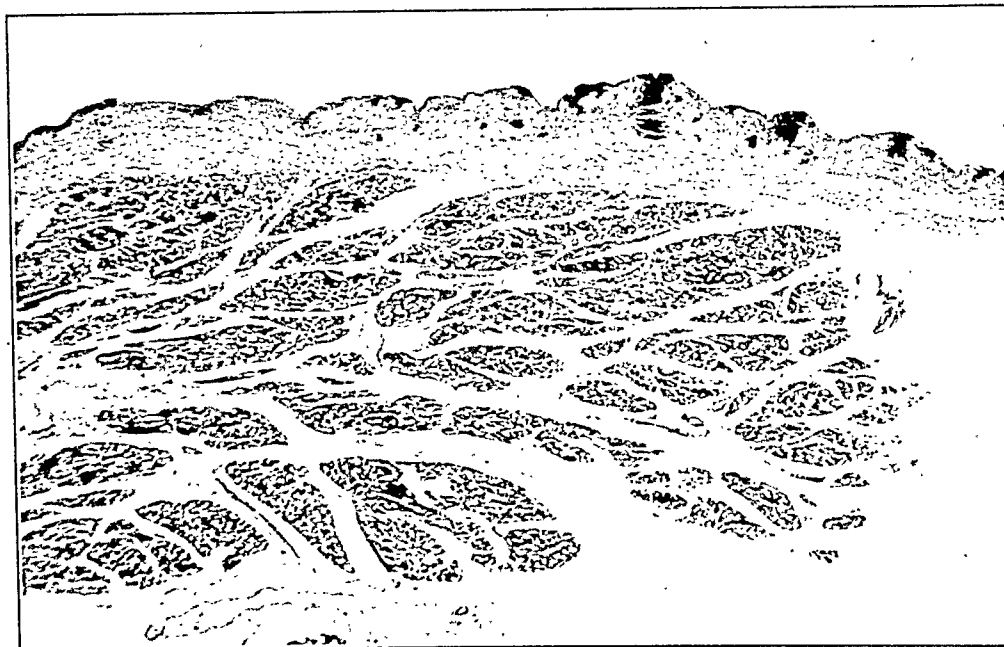


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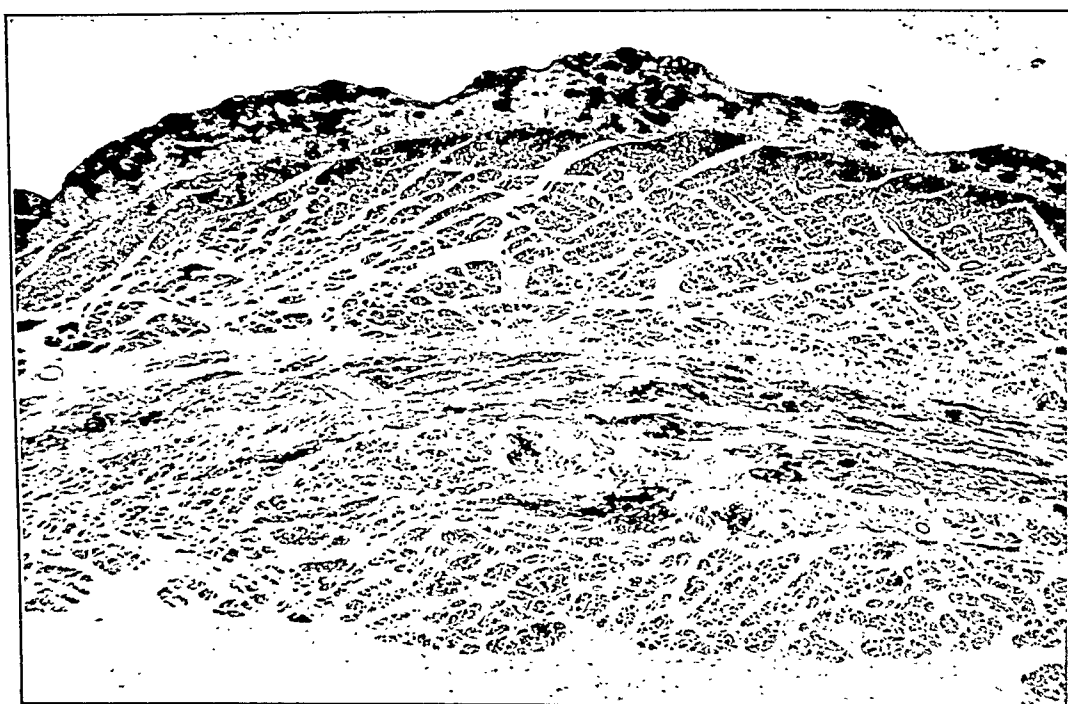
PLATE 86

FIGS. 8 and 9. Cross-sections of right auricle. Gentian violet stain. $\times 25$.

FIG. 10. Cross-section of right auricle. Gentian violet stain. $\times 125$.



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A CASE OF HODGKIN'S DISEASE IN A DOG *

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One hundred years have passed since Hodgkin¹ described clinically a disease of the lymphoid system that later acquired his name. During this period the literature has abounded with papers dealing with various aspects of this problem, perhaps most outstanding of which are the works of Wilks,² Sternberg,³ Reed,⁴ and Ziegler.⁵ The variety of clinical manifestations, the typical as well as the atypical histological types, the time honored discussion of the relation of this disease to tuberculosis, the problem of etiology, and lastly the question of whether or not this disease should be considered as a type of malignant tumor are all problems with which both clinicians and pathologists are only too familiar, and will not be discussed in this very brief report. With the literature as extensive as it is, it is quite possible that some papers may have been overlooked, but after a careful search I have been unable to find a single report of Hodgkin's disease occurring in the dog.

We have recently found a case, quite by chance, in a dog brought into the school for experimental purposes. The object of reporting this case is not to call attention to what would appear to be rather a curiosity, but to point out that a disease of lymph nodes, simulating Hodgkin's lymphogranulomatosis in man, may occur spontaneously in dogs.

The dog was of the large mongrel type and showed on examination a firm nodular swelling approximately 7 cm. in diameter along the right side of the neck. This swelling could be grasped in the hand and moved slightly, but it seemed definitely adherent to the overlying skin, which at one point showed a recent abrasion. A complete autopsy was performed which, with the exception of this mass in the neck, may be considered negative. Sections were taken from several areas in this mass and stained with a variety of stains to bring out cellular and intercellular detail; in addition a futile

* Received for publication September 6, 1933.

search was made for tubercle bacilli, both by direct smear and also in fixed tissue preparations.

Grossly the swelling consisted of a massive enlargement of the cervical lymph nodes, in the midst of which the individual nodes could not be defined. They were bound not only together, but also to the surrounding fascia and muscles. The mass varied in color, texture and consistence; some areas were soft, gray and homogeneous, others were gray, tough and fibrous. Still other areas, slightly caseous, suggested areas of necrosis.

Histologically, the normal architecture of the lymph nodes is almost entirely replaced by a very cellular type of granulation tissue showing a complex hyperplasia of endothelial cells, reticular cells, fibroblasts, lymphocytes, plasma cells, eosinophiles and, most characteristic of all, many large cells well recognized as the Sternberg or Dorothy Reed giant cells. These cells are both single and multinucleated with abundant, homogeneous blue-staining cytoplasm and large, round, oval or irregular nuclei showing not infrequently symmetrical and asymmetrical mitoses. Some areas are composed almost entirely of lymphocytes and plasma cells, others of reticular and endothelial cells supported by a delicate reticulum, still other areas much less cellular are sclerosed and made up of coarse bands of collagen fibers, isolating here and there typical Sternberg giant cells. The vessels are large and thin-walled, and in some areas where collections of cells have accumulated beneath the endothelium of the intima the lumina are almost obliterated. The capsule of the lymph nodes is infiltrated and the same confusion of cell types is seen out in the surrounding connective tissue and muscles.

The location of the lesion and the complexity of the histological picture, coupled with the presence of the characteristic Sternberg giant cells permit, from the standpoint of microscopy, the diagnosis of Hodgkin's granuloma almost with certainty.

The restricted localization of the disease in this case may lead one to doubt this diagnosis or to accept it with some hesitancy. Originally Hodgkin's disease was looked upon as a fairly disseminated lesion of lymphoid tissue, and probably even today most cases fall into this category. However, many cases are now diagnosed both by clinicians and by pathologists in which only a single group of lymph nodes are involved, or perhaps only a single organ or system of organs.

In a condition such as Hodgkin's disease, in which the etiology is still obscure, the final diagnosis must depend on the histological examination. Furthermore, as Sternberg⁶ has recently pointed out, in criticizing many of the so-called atypical types of Hodgkin's disease one must find the characteristic histological changes considered specific for the disease before one can establish a specific histological diagnosis.

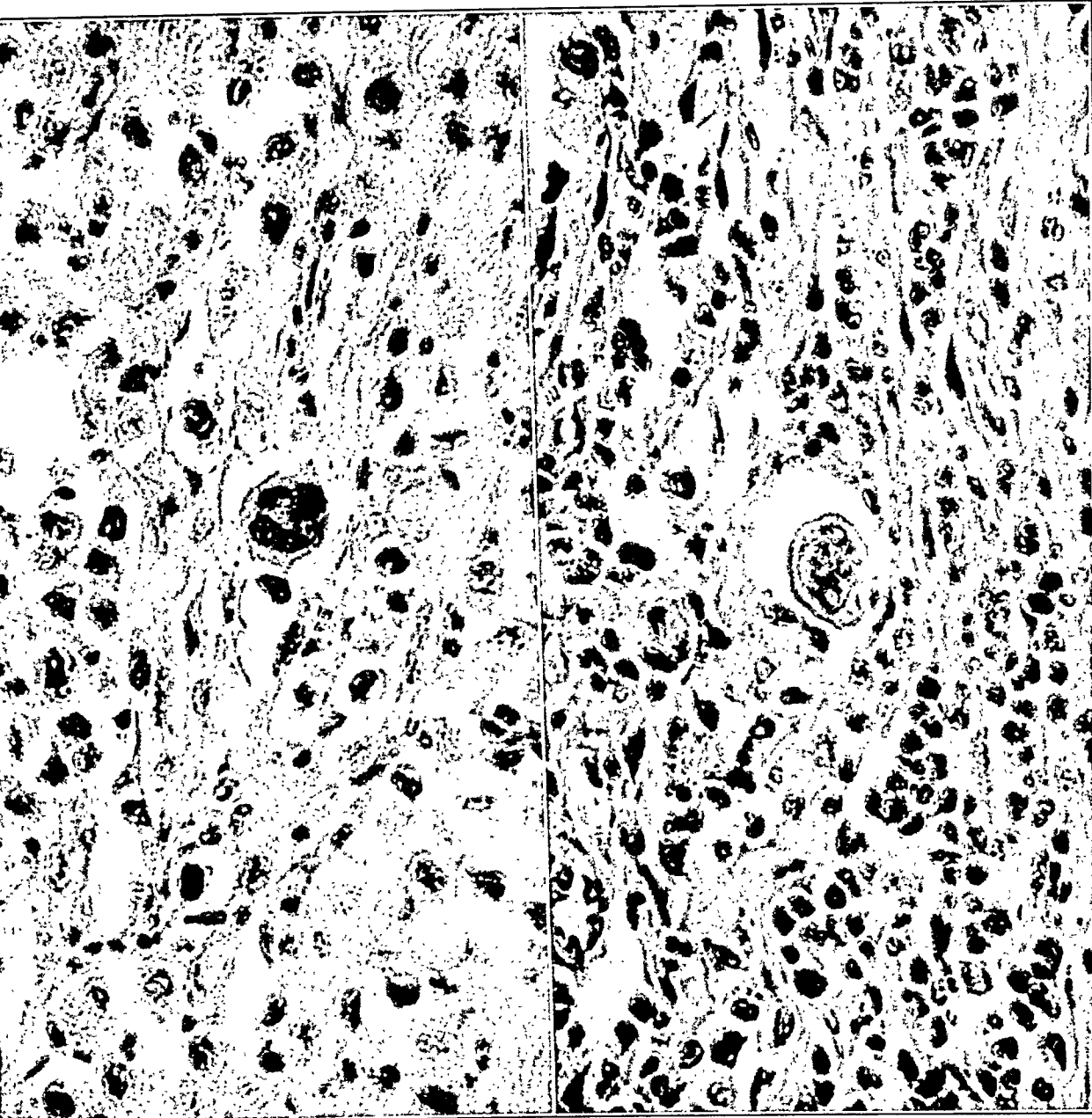
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DESCRIPTION OF PLATE

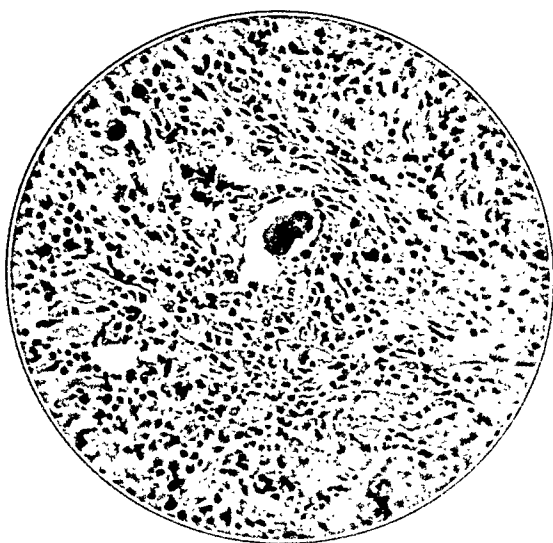
PLATE 87

- FIG. 1. Lymph node. In the center of the field is a large Sternberg giant cell surrounded by lymphocytes, reticular cells and fibroblasts. The background is composed of a delicate reticulum infiltrated with polymorphonuclear leukocytes, including an occasional eosinophil.
- FIG. 2. Lymph node. In the center of the field is a large Sternberg giant cell surrounded by lymphocytes, and an occasional large reticular cell. The stroma is abundant and is composed of interlacing bundles of collagen fibrils.
- FIG. 3. Lymph node. Section shows a large Sternberg giant cell in the center, surrounded by lymphocytes, fibroblasts, a few polymorphonuclear leukocytes and an occasional swollen reticular cell. The connective tissue stroma is fairly abundant.
- FIG. 4. Lymph node. Section shows four Sternberg giant cells, scattered lymphocytes, fibroblasts, an occasional polymorphonuclear cell, and a rather delicate reticulum stroma.

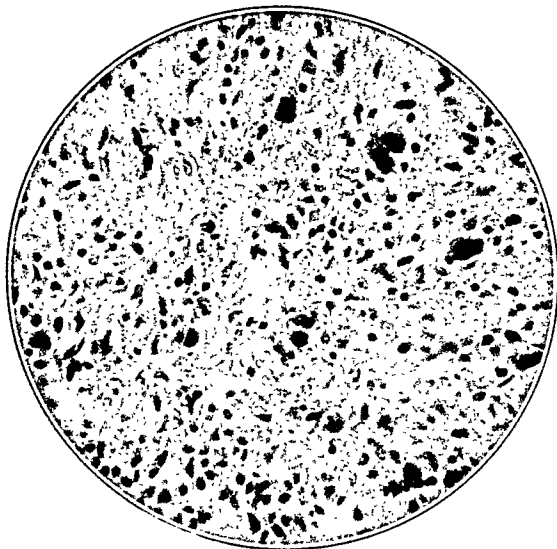


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MacMahon

Hodgkin's Disease in a Dog

PRIMARY CARCINOMA OF THE DUODENUM *

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Primary carcinoma rarely attacks the small intestine and when it does it is usually found in the duodenum or close to the ileocecal valve. From 1915 to 1919 inclusive Lahey,¹ Jefferson,² and Judd³ reported 24 cases of primary carcinoma in the small intestine distributed as follows: 11 cases were in the jejunum, 5 in the duodenum, 6 in the ileum and 2 showed multiple involvement. In 1925 Eusterman, Berkman and Swan⁴ reported 39 cases of primary carcinoma of the small intestine, of which 15 were in the duodenum.

The frequency with which carcinoma of the duodenum is found can be judged by the fact that between the years 1882 to 1931 inclusive there have been reported in the literature 6882 cases of intestinal carcinoma. Of these 147 or 2.13 per cent were in the duodenum. This is roughly 0.01 to 0.1 per cent of all postmortem examinations. The figures are usually given as 0.01 to 0.03 per cent.⁵ According to Brill,⁶ only 2.5 per cent of intestinal carcinomas are in the small intestine, being equally divided between the duodenum and the ileum.

For purposes of description carcinoma of the duodenum is divided into three groups, according to their anatomical position: (1) parapyloric, (2) peri-ampullary, and (3) prejejunal. They may be more conveniently classified as carcinoma of the first, second or third portion of the duodenum, or according to their relation to the major duodenal papilla as suprapapillary, peripapillary, and infrapapillary. Of these by far the most common type is the peripapillary, the most infrequent the infrapapillary or prejejunal.

The suprapapillary type has practically the same clinical picture as carcinoma of the pyloric end of the stomach and since it is relatively uncommon in this position and because of its close relation to the pylorus it is probably suspected less commonly than carcinoma of the pylorus. It usually encircles the duodenum, producing complete or almost complete stenosis with dilatation of all the bowel proximal to it, including the pyloric ring.

* Received for publication August 26, 1933.

The peripapillary is the most frequent type of carcinoma of the duodenum. It usually arises in or about the papilla as an infiltration and tends to encircle and extend along the duodenum. If it occurs in the upper part of the second portion of the duodenum or just above the major duodenal papilla, and not involving it, practically the same clinical picture will be produced as with carcinoma in the suprapapillary portion, or pyloric carcinoma. If it involves the papilla the flow of bile will be interfered with and jaundice or other complications will develop. If it is immediately below the papilla stenosis will gradually occur and give rise to obstruction with bilious vomiting.

It must also be remembered that carcinoma just above the papilla may produce the same clinical symptoms as that occurring on or in the papilla. This is due to the close proximity of the common bile duct to the wall of the intestine in this region, as a result of which it may gradually be involved and occluded by fibrous stricture or by direct extension of the growth into the duct. However, carcinoma above the papilla may be differentiated from carcinoma of the papilla because of the gradual development of persistent jaundice, in contrast to the intermittent type of jaundice usually seen with carcinoma of the papilla.

The term "carcinoma of the ampulla of Vater" is sometimes erroneously applied to carcinoma of the duodenum involving the papilla. It should be understood that by the former term is meant carcinoma arising inside the papilla, while the other type is on the surface of the papilla and of the intestinal mucosa. When the carcinoma arises on the surface of the papilla the flow of bile is interfered with, although not as a rule completely blocked. A change that may frequently follow on this and rapidly prove fatal is suppurative inflammation of the bile ducts.

The infrapapillary type of carcinoma is the least frequent. It usually is in close proximity to the ligament of Treitz and produces symptoms similar to those of intermittent intestinal obstruction. It resembles pyloric obstruction, except for the presence of bile and pancreatic juice in the vomitus.

It is not the purpose of this paper to enter into a discussion concerning the type of tissue from which the newgrowth arises. Suffice it to say that ulcer, aberrant tissues or fetal rests may be responsible, but because of the extreme difficulty with which the pathologist is

confronted in trying to differentiate them it seems sufficient at present to call these newgrowths adenocarcinoma, until better methods are developed which will enable us to be more accurate in a differentiation.

The following case we believe is worthy of reporting because it represents a primary carcinoma of the duodenum of the periampullary type occurring in close proximity to the papilla but not involving it.

REPORT OF CASE

Clinical History: The patient, C. P., a male negro, 45 years of age, was admitted to the Georgetown University Hospital with a history of progressive painless jaundice of 3 weeks duration, accompanied by belching, heartburn, sour stomach and vomiting on one occasion. There had been progressive weakness, loss of weight (18 pounds), and diarrhea for 3 weeks, but no bloody or tarry stools had been present. Previous health up to time of onset of present symptoms was excellent.

Physical examination disclosed a marked icterus of the sclerae, and pronounced weakness. The abdomen was slightly distended and a small mass about 8 cm. in diameter was felt in the gall-bladder region.

The urine contained a large amount of bile, albumin and many casts. The blood contained 68 per cent hemoglobin, 3,330,000 red blood cells and 7400 white blood cells, of which 80 per cent were polymorphonuclear. The Wassermann reaction was negative. The van den Bergh test was direct, prompt; indirect 60 mg. On X-ray study a dilated stomach and duodenal cap were seen. Following barium injection there was 60 per cent retention, and vomiting became markedly aggravated.

The patient was operated upon a week after admission. A mass was found in the region of the head of the pancreas. A posterior gastrojejunostomy and cholecystotomy were performed for duodenal obstruction, believed to be due to a duodenal carcinoma. Death occurred 2 days later as the result of toxemia and bronchopneumonia.

POSTMORTEM EXAMINATION

At autopsy the liver weighed 1890 gm. It was markedly jaundiced and very soft, making the normal markings indistinct. The gall-bladder was normal but contained a rubber tube drain sutured in the fundus. The common duct was markedly dilated and obstructed in its lower third (Fig. 1). Dissection of this obstructed portion of the duct disclosed a patent lumen with a few small nodules in the mucosa measuring about 2 mm. in diameter. This part of the common duct was firmly adherent to the adjacent portion of the duodenum and was surrounded by dense fibrous tissue. The duodenal wall at this point was firm and presented a hard ring

around the entire circumference. The bowel proximal to this ring was greatly dilated and distal to it was collapsed. On opening the duodenum a circular area of induration about 5 cm. in diameter was present 2 cm. above the major duodenal papilla. The induration extended through the wall of the bowel, producing a marked thickening and forming a ring, as mentioned above. The extraduodenal portion of the common bile duct was involved in this mural thickening and became obstructed by it. No evidence of metastasis or glandular involvement was found.

The head of the pancreas was normal and without evidence of involvement.

Other important findings at autopsy were a well developed bronchopneumonia and complete transposition of the colon.

The most important microscopic findings were a bronchopneumonia and a low grade adenocarcinoma in the area of induration in the duodenum. The entire wall of the duodenum in the region of induration was invaded by large, irregular, well developed glandular acini composed of tall columnar epithelium. The wall of the common duct was invaded by similar glandular structures. Considerable fibrous tissue was present, intermingled with the carcinomatous growth (Fig. 2).

SUMMARY

Statistics showing the frequency of duodenal carcinoma are presented, together with salient factors that are necessary when one attempts to differentiate neoplasms of the suprapapillary, peripapillary, and infrapapillary areas of the small bowel.

A case report is also given in which a low grade adenocarcinoma of the duodenum was found 2 cm. above the major duodenal papilla. The clinical signs and symptoms simulated those of carcinoma of the head of the pancreas and carcinoma arising in the major duodenal papilla.

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DESCRIPTION OF PLATE

PLATE 88

FIG. 1. Photograph of the gross specimen dissected along the course of the common duct to determine the point of obstruction.

A, represents the dilated common duct.

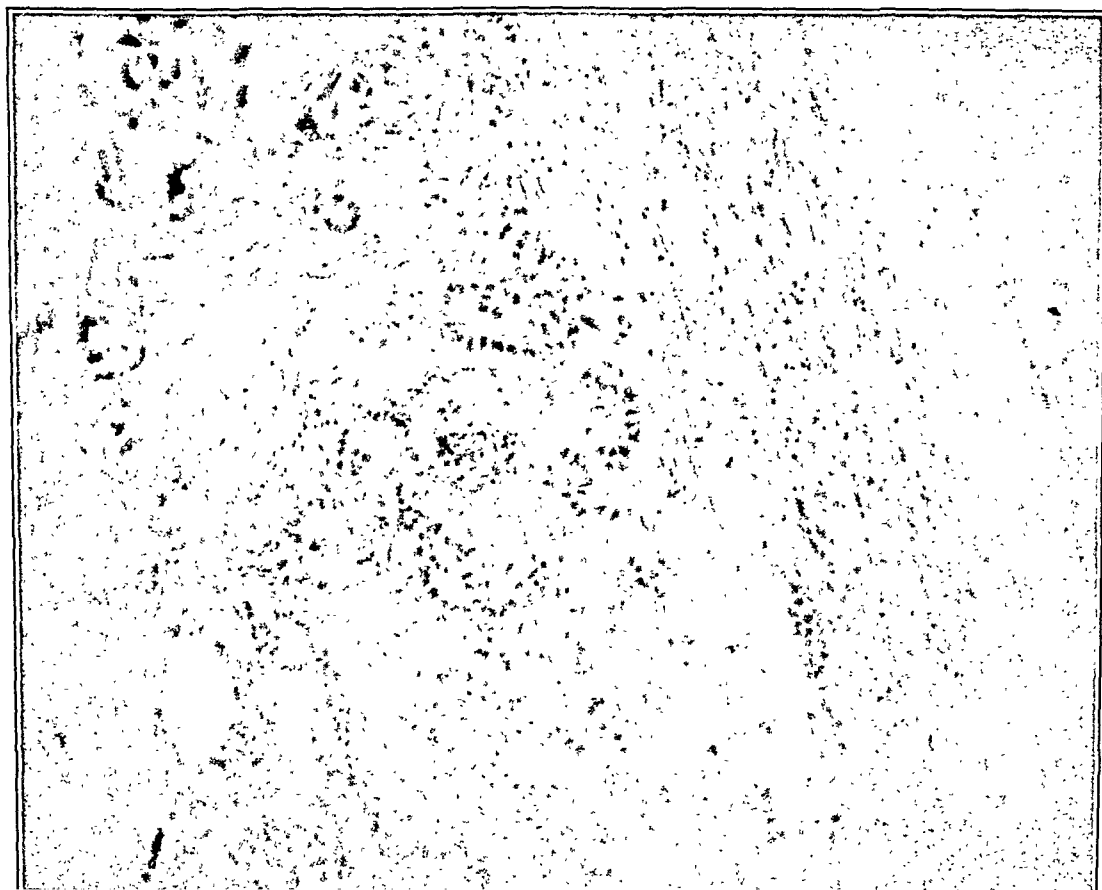
B, shows the point of constriction of the duct at which obstruction occurred. The constriction was due to the carcinoma encircling the duct.

C, at this point can be seen the marked thickening of the duodenal wall produced by the carcinoma and fibrous tissue, as seen in Fig. 2.

FIG. 2. Photomicrograph of a section taken at point C in Fig. 1. In it can be seen differentiated acini of columnar epithelium lying in a loose fibrous stroma. Similar acini were found throughout the entire thickness of the bowel. It represents a low grade adenocarcinoma.



I



ADDENDA TO A THEORY OF PIGMENTED MOLES

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MIESCHER'S PAPER

After our paper on the theory of pigmented moles¹ had been sent to press Professor Masson, visiting this laboratory, called our attention to the confirmation of his observations by Miescher² in a recent volume of Jadassohn's great Handbuch. Miescher's paper is undoubtedly the best written and best illustrated discussion of pigmented moles that has appeared in recent years. Like Masson, Stout, Ewing and Foot, Miescher finds neural plexuses and groups of tactile corpuscles in many pigmented moles and he justly insists that in order to demonstrate these structures the moles must be properly fixed and stained. He announces a more detailed study of the neural structures in pigmented moles by Miescher and von Albertini, to appear in *Virchows Archiv*, 1933 (not yet published).

From Miescher's paper we select this significant paragraph: "In their structure and in the relation of the different tissue elements to one another, pigmented moles differ so absolutely from all known microscopic pictures that it is difficult to understand their nature and to comprehend their significance." (See page 1015.)

It is true that if we restrict our studies, as Miescher has done and as every other histologist has done, to mammalian skin, the structure of a pigmented mole is absolutely incomprehensible. But if we look at sections of amphibian or reptilian skin we shall see each puzzling feature of the human pigmented mole fall naturally into place as part of a normally functioning amphibian or reptilian tactile terminal.

Referring to our own paper, compare the impression produced by our Figures 1 to 5, the neural structures in human pigmented moles, with Figures 7 and 8, sections through normal reptilian tactile spots. In Figures 1 to 5 the groups of innervated tactile cells in the derma, the intermingling columns of schwann cells, the threadlike terminal nerves running in all directions, the irregular accumulation of pigment, — all this in human skin is, as Miescher says, a meaningless

jumble. In the reptilian skin of Figures 7 and 8 the intermingling of exactly these same structures constitutes a well ordered and highly efficient tactile terminal.

A CHRONOLOGICAL PARALLEL

In connecting the ontogeny of the pigmented mole with that of the hair follicle we explain the hitherto puzzling phenomenon of hereditary and "congenital" pigmented moles appearing at intervals during the entire life of the individual. The periodical eruption of pigmented moles parallels the periodic outbursts of new hair follicles. It is well known that pigmented moles appear most abundantly at birth or shortly thereafter, next at puberty, and then in diminishing numbers scattered through later years. This is precisely the sequence of activity in the extension of hair follicles over new areas. Obviously the factors, probably of hormonal nature, which induce the formation of new groups of hair follicles (mammalian tactile organs) operate at the same time on those aberrant groups of cells that already have been determined for the formation of the reptilian type of tactile organ — the pigmented mole.

In accord with this observation are data from experimental embryology which show that the time order of the events of segregation, *i.e.*, determination of ultimate potencies of cell groups, is quite independent of the actual organogenesis, which often occurs much later and whose time of incidence is dependent upon numerous factors operating in the developing and functioning organism.

In addition to the chronological parallel between hair follicle and mole there is another, that of pigment formation. Of all derivatives of the epidermal ectoderm, normal or pathological, the hair matrix and the pigmented mole alone exhibit regularly and prominently the melanogenic properties of the surface epithelium.

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THE AMERICAN JOURNAL OF PATHOLOGY

VOLUME X

MAY, 1934

NUMBER 3

THE EFFECT OF SINGLE AND MULTIPLE DOSES OF THE PARATHYROID HORMONE ON THE CALCIFICATION OF THE DENTIN OF THE RAT INCISOR*

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INTRODUCTION

The specific effect of the parathyroid hormone on calcium metabolism has, with few exceptions, been generally accepted. The dentin of the rat incisor has been found to be extremely sensitive to changes in calcium metabolism. Erdheim,¹ in his parathyroid auto-transplant experiments on rats, demonstrated a calcium-free stripe of dentin that corresponded in position and width with the time and duration of the interval that elapsed between the removal of the parathyroid and the "taking" of the transplant. Schour and Ham² studied the effect of single doses of ergosterol and parathyroid hormone on the dentin of the rat incisor. They were able to correlate definite histological reactions with the blood calcium findings and, particularly, with the periods of rise and fall of the blood calcium. It was, therefore, found advisable to investigate further the effect of single and multiple injections of varying doses of parathyroid hormone on the calcified tissues of the rat incisor, particularly the dentin, and to correlate these effects with the blood calcium changes and the alterations in other tissues in the body which have been fully described in a previous report.³

* The preparation of a portion of the hormone used in this report was aided by a grant from the committee on scientific research of the American Medical Association.
Received for publication November 23, 1933.

TABLE I

*Data on 20 Rats that Were Given Single and Multiple Injections of Parathyroid Hormone,
Arranged in Order of Number of Injections¹*

Rat No.	Weight gm.	Duration of experiments days	Dosage units and times injected	Time between last injection and death hrs.	Blood serum calcium mg. per 100 cc.	Necrosis of kidney	Histological changes in the dental tissues					
							Enamel hypoplasia	Dentin			Alveolar bone	
								Calcification		Vascular or cellular inclusions	Predominance of osteoclasts	Fibrous marrow spaces
								Poor immediately after first injection	Excessive subsequent to first injection			
316	91	1	50 (1X)	10	13.0	-	-	+	-	-	-	-
317	107	1	75 (1X)	10	13.6	+	-	+	-	-	-	-
318	118	1	100 (1X)	10	12.6	+	-	+	-	-	-	-
320	86	1	100 (1X)	10	9.2	-	-	+	-	-	-	-
321	125	2	150 (1X)L	48		++	-	+	+	-	-	-
329	80	3	140 (1X)	72	11.2		-	-	-	-	-	-
380	125	2	150 (1X)L	48	14.8	++	-	-	-	-	-	-
309	125	2	45 (2X)	10			-					
336	130	3	25 (3X)L	10	13.7	-	-	Slight	Slight	-	Slight	-
337	143	3	25 (3X)L	10	11.2	-	-	Slight	Slight	-	Slight	-
338	135	3	25 (3X)L	10	12.4	-	-	-	Slight	-	Slight	-
342	113	3	50 (3X)L	10	17.1		-	+	+	-	Slight	-

TABLE I — Continued

331	122	10	50 (3X)L	19	12.1	++	-	+	+	+	+	Slight	-
332	110	3	50 (3X)L	19	15.2	++	+	+	+	+	+	+	-
333	120	3	50 (3X)L	19	14.4	++	+	+	+	+	+	+	-
334	125	3	50 (3X)L	19	15.5	++	-	+	+	+	+	-	-
370	120	3	50 (3X)*	19	10.8		-	-	-	-	-	-	-
371	105	3	50 (3X)*	19	10.7		-	+	+	+	+	-	-
301	104	6	15 (4X)	72	13.0	+	+	+	+	+	+	Slight	-
302	118	5	22 (4X)	19	14.8	+	-	+	+	+	+	-	-
303	147	4	30 (4X)	19		++	-	+	+	+	+	-	-
324	130	7	50 (4X)	19	17.0	++	+	+	+	+	+	+	+
323	146	10	50 (9X)	19			+	+	+	+	+	+	+
326	145	15	10 (12X)	19	11.4	-	-	-	-	-	-	-	-
327	130	15	10 (12X)	19	10.8	-	-	-	-	-	-	-	-
307	88	16	18 and 36 on alternate days (12X)	19	13.7	++	-	+	+	+	+	-	-
308	117	13	23 and 46 on alternate days (12X)	19			-	+	+	+	+	Slight	-
306	85	18	17 and 34 on alternate days (13X)	19	15.4	++	+	+	+	+	+	+	+
305	115	15	17 and 34 on alternate days (14X)	19	22.0		-	+	+	+	+	+	-
			** (15X)										

L = parathormone

* = inactivated hormone

** = this animal received 17 to 23 units for the first 3 days, the following period from 23 to 46 units, and finally 24 hours before being killed 75 units. A total of 450 units was given. The animal was very sick the day following the last injection.

¹ The following bracket figures represent the numbers of the animals and their table in the preceding report: 301 (1, T. 3), 302 (2, T. 3), 306 (3, T. 3), 316 (5, T. 3), 317 (4, T. 3), 318 (6, T. 3), 320 (7, T. 3), 322 (1, T. 2), 324 (2, T. 2), 326 (3, T. 2), 327 (4, T. 2), 331 (5, T. 2), 332 (6, T. 2), 333 (7, T. 2), 334 (8, T. 2), 336 (9, T. 2), 337 (10, T. 2), 338 (11, T. 2), 370 (12, T. 2), 371 (13, T. 2), 374 (19, T. 3), 380 (20, T. 3).

METHOD AND MATERIAL

This study is based on the same material that McJunkin, Tweedy and Breuhaus³ used in their investigation of the effects of the parathyroid hormone on the parathyroids and on the tissues of the rat.

The series consisted of 29 rats weighing from 80 to 147 gm. The doses of parathyroid hormone varied from 1 to 15 in number, and from 10 to 150 units in quantity. In animals that were given repeated doses the interval between the injections varied from 24 to 72 hours (Table I). The animals were killed usually 19 hours after the last injection. Seven controls from the same colony and within the same age limits as the experimental animals were studied histologically. In addition the histological data of the dental tissues of 104 normal rats used in other studies conducted by the senior author were available.

Histological Methods: The heads of the animals were fixed in 10 per cent formalin immediately after death. A midsagittal section of each head was made to facilitate X-raying. The teeth and their investing tissues were then dissected further in order to facilitate fixation and histological preparation. The tissues were washed, decalcified in 5 per cent nitric acid, treated with sodium sulphate, washed, dehydrated and embedded in celloidin. The sections were cut and mounted in serial order and stained with hematoxylin and eosin. A small percentage of the sections was also stained with iron hematoxylin and Mallory's connective tissue stain. Midsagittal and transverse sections were prepared in each case. In a number of animals ground sections of the hard tissues alone, and ground sections of both the hard and soft tissues, stained with hematoxylin and eosin or with alizarin, were also prepared.

Chemical Methods: Before the animals were killed blood was drawn by cardiac puncture and the calcium analysis made, in most instances, on a 1 cc. sample of serum. The Kramer-Tisdall method, as modified by Tweedy and Koch, was employed.

The blood serum calcium of 7 rats picked at random from the same colony and on the same stock diet ranged from 10.62 to 11.60 mg. per cent. The diet consisted of fox chow (commercial preparation) supplemented by lettuce, cheese and lean meat.

The hormone preparation designed "L" in the tables is parathormone (Eli Lilly and Company). The preparation not so designated was prepared by the method of one of us (Tweedy⁴) and standardized by Collip and Clark's procedure.⁵ The acid alcohol inactivated hormone preparation is described by Tweedy and Torigoe⁶ in a separate publication.

HISTOPHYSIOLOGY OF DENTIN OF NORMAL RAT INCISOR

Dentin, like bone, consists of an organic matrix substance which becomes calcified. It is apposed at the pulpal surface in the form of layers, which in the adult rat grow forward at the same rate as that of eruption. The position and width of a given layer indicate the time it was formed and its duration.

The dentin matrix is calcified in the form of globules that are normally small and numerous and so close together that there results

a uniformly calcified tissue. But even in the normal dentin the successive layers of dentin are not equally well calcified. Well calcified layers alternate more or less regularly and rhythmically with imperfectly calcified layers, so that there arises a stratification in the dentin, especially toward the incisal edge (Schour⁷). There is a distinct smooth boundary between the matrix that was laid down last, called predentin, and the calcified dentin matrix (Fig. 1).

The staining reaction of dentin to hematoxylin and eosin is a dependable, although not a perfect, indicator for its state of calcification (Schour and Ham⁸). The dentin that takes on the hematoxylin homogeneously has been generally and, in the authors' opinion, correctly accepted as being well calcified. The dentin that does not take the hematoxylin stain has been generally regarded as uncalcified dentin and has been named predentin. The latter, as a rule, takes the eosin stain but, frequently enough as careful observation reveals, this layer is found to be entirely or in part devoid of the eosin stain also. There are, therefore, on the basis of the hematoxylin-eosin reaction, two types of predentin which, for convenience, will be named early and late predentin.

The early predentin is found next to the pulp and appears first. It reacts neither to the eosin nor to the hematoxylin stain and although it already contains dentinal tubules it reminds one of the *membrana preformativa* (Raschkow) of the dental papilla found in the tooth germ stage which just precedes the formation of the hard tissues. It is pale gray with the iron hematoxylin and pale blue with the Mallory connective tissue stain.

The late predentin takes the eosin stain. It is dark gray with the iron hematoxylin and dark blue with the Mallory connective tissue stain.

In given fields either stage may be absent. When both are present the late predentin is often found to intervene in the form of a narrow ribbon-like eosin stripe between the early stage and the calcified dentin. When only one stage is present it occupies the entire space between the pulp and the calcified dentin and the boundary between the latter and the particular stage is smooth and linear.

The specific staining of the dentin matrix of the rat incisor for argyrophil and collagenous fibers (Orban⁹) will show whether the early predentin represents a precollagenous matrix and the late predentin a collagenous one or not.

It is possible that the eosin-staining dentin is not calcium-free as has been generally supposed, but represents a preparatory stage of calcification. Whether the different staining reactions of the so-called predentin and the calcified dentin lie in a difference in the quantity of inorganic salts or in a difference in the combination of inorganic salts is not known.

HISTOPATHOLOGICAL FINDINGS

A few of the representative experimental animals will be described in detail.

Effect of Single Doses

The rats that were killed 24 hours after a single injection of 50, 75, or 100 units of parathyroid hormone, respectively, show an alteration in the calcification of the dentin formed during the experimental period (Table I). This disturbance is confined to the predentin layer which is slightly wider than normal (20 to 25 μ) and which consists of an eosin-staining portion that borders smoothly against the hematoxylin-staining dentin of the preoperative period, but irregularly against the portion that is adjacent to the pulp and that stains with neither hematoxylin nor eosin. This disturbance becomes aggravated with the increase in the number of units.

Figure 2, taken from Rat 317, which was given 75 units of parathyroid hormone, shows the eosin-staining predentin which is irregular where it faces the early or non-eosin-staining predentin. The latter, in addition, is not homogeneous but contains isolated eosin-staining globules.

Figure 3, taken from Rat 318, which was given 100 units, shows a more aggravated disturbance. The non-staining predentin is sprinkled with a greater number and a smaller size of eosin-staining globules. In addition, the dentin that was laid down just previous to the time of the injections takes a deep hematoxylin stain and is preceded by a lighter eosin-staining stripe.

Figure 5, taken from rat 374, which was given one dose of 150 units of parathyroid hormone, shows in the dentin that was formed during the 48 hours of the experimental period (about 30 to 35 μ) first an eosin-staining dentin and then pulpally a deep hematoxylin-staining stripe. The latter in some places is replaced and modified so that it does not react to hematoxylin or eosin. On the other

hand, Rats 376 and 380, which were also given single doses of 140 and 150 units respectively, showed no histological changes in the dentin.

Effect of Multiple Doses of Parathyroid Hormone

3 Injections of 25 Units Each (Rats 336, 337 and 338): The dentin situated within approximately 30 μ from the pulp takes a deeper hematoxylin stain (Fig. 4). There is, labial to this dentin, an indication of a lighter eosin stripe. Osteoclasts are seen more readily than normal in the labial alveolar bone.

3 Injections of 50 Units Each (Rats 322, 331 to 334, 370 and 371): The first 3 rats that were given 3 injections of 50 units each show a similar and typical reaction. The latter is characterized by an eosin-staining layer that corresponds with the dentin formed immediately following the first injection and a deep hematoxylin-staining layer that corresponds with the dentin laid down during the time of the 2nd and 3rd injections.

Some portions of the tooth, particularly the midregion in longitudinal sections and the lateral surfaces in transverse sections, show a disturbance and deviation from the typical reaction in respect to the staining reaction of the dentin hypercalcified during the period comprising the last 2 injections. In these regions the deep hematoxylin reaction may be absent entirely or in part, so that the corresponding dentin stains only with eosin or stains neither with eosin nor hematoxylin. The dentin formed within 24 hours of the time of death shows an eosin or non-staining reaction. The entire dentin involved extends from 35 to 46 μ .

In Rat 331, in which the experiment extended over a 10 day period, the reaction is similar but is found to be extended over a wider portion of the dentin and is less disturbed than in rats in which the same number of injections followed in closer succession. The boundary between the pulp and the predentin is wavy toward the anterior end of the pulp and the stratification is similar to that shown in Figure 9. Rats 370 and 371, which were injected with the inactivated parathyroid hormone preparation, showed a normal histological picture.

4 Injections of from 15 to 50 Units Each (Rats 301 to 303 and 324): Rat 301 will be taken as a representative of this group and the findings will be described in greater detail.

The enamel surface shows a region of severe enamel hypoplasia (Fig. 6). The latter is situated at approximately 1.6 mm. from Hertwig's sheath. The dentin next to this enamel defect is taking the eosin stain and marks the beginning of a stripe that can be traced toward the incisal edge (Fig. 6). The disturbance in the calcification of this stripe is least prominent in the posterior region (Fig. 7). Over the middle portion of the tooth this eosin stripe is $15\ \mu$ in width and is followed pulpally by a wider stripe of dentin, which stains deeply with hematoxylin (Fig. 8). In this figure the width of the dentin from the outer end of the eosin stripe to the pulp is $90\ \mu$. This portion of the dentin represents the amount laid down during the duration of the experiment and approximates the width of dentin that would be laid down in a period of 6 days in a normal animal of similar age.

Thus the dentin laid down immediately following the first injection is poorly calcified and remains in this state. The dentin laid down subsequently is overcalcified and continues to be so throughout the remainder of the experimental period, except for the apparently normal predentin next to the pulp. The overcalcified stripe shows some variations. It sometimes takes a pale blue color next to the eosin stripe. In the posterior portion of the tooth its overcalcification is less intense (Fig. 7) than in the middle portion (Fig. 8). In the more anterior region it shows prominent stratification, and in addition vascularization (Fig. 9).

Rats 302 and 303 show similar pictures, except that the changes involve a smaller area of dentin (Fig. 12), since the experiments lasted only 5 days and the pattern of the reaction is severely disturbed in the midregion of the tooth. Figure 12 which is taken from the midregion shows a stripe that is quite pale blue in color. Rat 324, which had 4 injections, also showed a reaction that for the most part was similar to that observed in Rat 301. Vascular and cellular inclusions in the dentin are prominent (Fig. 15).

Multiple Injections of Parathyroid Hormone Greater than 5 in Number (Rats 305 to 308, 323, 326 and 327): Rat 323 shows changes similar to those of Rat 301. In addition, vascular and cellular inclusions in the dentin are more prominent. There is also prominent engorgement of the blood vessels in the pulp. The dentin in the molar shows a reaction in the form of a persisting eosin stripe which is followed by a deeper hematoxylin-staining dentin band and predentin (Fig. 14).

Rats 326 and 327, which were given 12 daily injections of 10 units, each show a normal histological picture. The stratification in the dentin is readily observed in the dentin nearer to the pulp but is within the range of normal variation.

Rat 307, which was also given 12 daily injections but of higher unitage (alternately 18 and 36 units), shows prominent and abnormal stratification like that seen in Figure 13, depicting the effect in Rat 306. The dentin shows vascularization in some places. Rat 308 shows some stratification which is irregular but, as a whole, normal.

Rat 306 shows prominent, irregular stratification consisting of eosin and hematoxylin-staining stripes with pale blue-staining bands interspersed. The area of stratification is $290\ \mu$ in width and approximates the amount of dentin that would be laid down in a normal animal of similar age in a period corresponding with the duration of the experiment (18 days). The dentin laid down previous to this width is normal (Fig. 13). There is severe enamel hypoplasia. The alveolar bone shows a predominance of osteoclasts and a fibrous change in the bone marrow (Fig. 11). Compare with Figure 10 which is taken from a corresponding section of control Rat 309. The dentin in the molars also shows an abnormal picture similar to that shown in Figure 14. Rat 307 shows a similar but less intense reaction than is seen in Rat 306.

The findings in the ground sections confirm those in the decalcified preparations.

DISCUSSION

Effect of Single Injections: The layer of the predentin that is ready to be calcified at the time of the injection responds immediately to the experimental condition. For convenience this portion of the dentin will be called the primary hypocalcified stripe. It is characterized by its failure to stain with hematoxylin and by its reaction with eosin either in a homogeneous manner or, in the case of higher dosage (75 to 100 units), in the form of globules (Figs. 2 and 3). It is commonly accepted that an irregular boundary between calcified dentin and predentin in the form of globular projections is an indication of disturbed calcification. In the experimental condition of single high doses, also, we have a corresponding disturbance in the form of an irregular boundary, only the latter is situated between the late and early predentin. Apparently we are

dealing with a disturbance and retardation of the normal progressive transformation of early predentin into late predentin, and of the latter into the fully calcified dentin.

The findings in Rat 374, which was given an injection of 150 units and which was allowed to live 48 hours (Fig. 5), show that the primary injection stripe, which on the basis of its position belonged to approximately the first 24 postinjection hours, remained more or less unchanged. However, the dentin that corresponded to approximately the second 24 hour postinjection period, and which may be called the secondary hypercalcified stripe, showed a tendency to overcalcification. In this case of a single high dose the effect appears to be similar to that observed by Schour and Ham² in single 40 unit injections, but accelerated and more irregular. While they found overcalcification only during the approximately second 50 hour postinjection period, in Rat 374 this condition appeared during the second 24 hour postinjection period and showed in parts defective calcification as well. The dentin, thus, acted as if, following the high dose of 150 units, the blood calcium curve both reached its peak and declined twice as fast as was the case with the dosage of 40 units.

The left kidney was removed 48 hours before death from Rats 374, 376 and 380 for microscopic examination. The absence of response in the dentin in Rats 376 and 380 may be accounted for on the basis of differences in the degree of shock produced by the operative procedure.

Effects of 3 Injections: The reaction varies with the dosage and the duration of the experiment. The sequence of events in the rats that received 25 unit doses (Fig. 4) is similar to that seen following 50 unit doses, except that following the higher doses the overcalcification of the secondary stripe is somewhat irregular and lacks uniformity, and the primary stripe associated with the first effect of the first injection is more prominent.

Rat 331, in which the 50 unit injections were spread over a longer period of time (10 days), showed a reaction that was less intense than that of the animals of shorter experimental life and which, of course, extended over a wide width of dentin corresponding to the amount laid down in 10 days.

The absence of reaction in the rats that were given the inactivated parathyroid hormone preparation gives conclusive proof that the effects observed were due to the parathyroid hormone and not to

any foreign protein impurities. Further evidence was obtained from 2 rats that were given 2 injections of kidney substance and that showed a normal picture in their dentin. An extensive series of rats which were treated with other tissue extracts showed no reaction in the dentin (Schour¹⁰).

Effect of Multiple Injections (More than 3): The reaction follows the general pattern observed in the experiments with fewer injections, but shows more severe disturbances in the calcification of the dentin formed toward the latter part of the experiment. These alterations consist of vascular and cellular inclusions, irregular outline of the pulpal surface, and greater prominence in the interspersation of stripes which appear to have remained in the early predentin stage (Fig. 15).

Enamel Hypoplasia: The fact that in 5 rats enamel hypoplasia is found at the level where the primary injection stripe begins shows that the enamel defect started at the time of the first injection (Fig. 6). Apparently at that time the disturbances in calcium metabolism were so severe and intense that both enamel and dentin suffered an acute injury. The fact that the enamel hypoplasia began at the time of the first injection is confirmed in some rats by the measurement of the distance between the enamel hypoplasia and the Hertwig's sheath. The distance corresponds closely with the extent of the eruption of the teeth during the experimental period. With the exception of Rat 301, each rat that showed enamel hypoplasia also showed an abnormal increase in the number of osteoclasts. Since the enamel hypoplasia began as a rule at the time of the first injection and in no apparent correlation with the amount of the dosage, it is possible that the susceptibility of the individual rats played an important rôle in the reaction of the enamel and the enamel epithelium. The absence of enamel hypoplasia in the rats that were given single doses of large unitage may be explained on the basis of their brief postinjection life (19 to 48 hours). It is possible that these injections produced in the ganoblasts an immediate injury which, however, cannot be recognized histologically until the cells reach a more highly differentiated and functional state.

Summary of Dentin Reaction: In spite of considerable individual variations in respect to the intensity of the reaction the principal changes are similar. The dentin is disturbed in respect to the quantity and rhythm of calcification. Our present histological methods

are inadequate to point out to what extent the quality of calcification may be disturbed as well.

The extent and type of the disturbance varies not only with the amount and frequency of the injections, the duration of the experiment, the time interval between successive injections and between the last injection and the death of the animal, and to a limited extent with the individual animals, but also with the relative chronological position of the dentin in respect to the time of the injections. In practically all cases the dentin of the postinjection period shows: (a) the primary stripe which is characterized by incomplete and deficient calcification and which in its position corresponds with the time immediately following the first injection (see all figures except 10 and 11); and (b) the secondary stripe which is characterized by overcalcification and which corresponds in its position with the time subsequent to approximately the first 24 hour postinjection period (Figs. 5, 8 and 12), (Table I).

The findings in the "multiple injections" experiments show that the calcification of late predentin is more or less permanently disturbed, so that the primary injection stripe is constant and does not disappear.

The secondary stripe varies in width with the duration of the experiment, and in its pattern with the number and unitage of the doses. In the rats that were killed within 24 hours after a single injection the secondary stripe is naturally absent (Figs. 2 and 3), (Table I).

The primary stripe may be regarded as an immediate acute reaction to the first injection. The secondary hypercalcified stripe may be regarded as a chronic reaction and indicative of an effort at healing.

Both stripes vary, within limits, in their width and staining reaction with their position in respect to the anteroposterior extent of the tooth and in respect to their distance from the pulp. Any given reaction is found to be as a rule most prominent in the midportion of the incisor and least prominent in the more posterior portion, and appears to be reduced with the increasing distance from the pulp. The histological study was centered on the calcification of dentin. The possible changes in the odontoblasts have not been studied.

Theories on the Mechanism of Parathyroid Hormone Action: The mechanism by which there results a mobilization of calcium in the

blood following the administration of parathyroid hormone into a reactive animal has not been established. Three theories, however, are prominently in the foreground of the controversial literature on this subject. Briefly stated they are as follows. (1) The administration of parathyroid hormone makes the blood plasma a better solvent for the calcium compounds of the blood or other tissues. For example, Greenwald¹¹ was the first to suggest that there circulates in the plasma a substance "x," which is identical with parathyroid hormone, or is formed under its influence, and which unites with calcium ions to form an undissociated compound, thus reducing the concentration of calcium ions in the plasma and permitting the liberation of more calcium ions from bone. It is evident that this theory depends upon the conception that the plasma behaves as if in equilibrium with tricalcium phosphate. (2) The parathyroid hormone lowers the renal threshold for the inorganic phosphate, so that plasma phosphate decreases and the plasma calcium is enabled to rise. This view has been put forward by Albright and co-workers.¹² The basic idea of this theory is that the plasma inorganic phosphate and calcium bear a reciprocal relation to one another, and behave as if in equilibrium with tricalcium phosphate. (3) The parathyroid hormone directly stimulates the cellular elements in the bone to increased osteoblastic or osteoclastic activity. This view has been advanced by Selye.¹³

Space does not permit a critical discussion of the controversial literature dealing with these theories. The senior author and his associate in a recent paper⁸ have discussed their reasons for believing that the experimental findings on which the last theory is based have been incorrectly interpreted. We also feel that the destructive critical evidence advanced by Thomson and Pugsley¹⁴ casts grave doubts on the validity of the second theory. While we are not prepared to accept the first theory we feel that the experimental findings in this work fit in best with the conception that the parathyroid hormone controls a definite fraction of the plasma calcium.

Possible Basis for the Dental Changes in Experimental Hyperparathyroidism: We believe that, fundamentally, the pattern in the dentin represents its response to the changes in the calcium and phosphorous metabolism induced by the injections of the parathyroid hormone.

The fact that in single administrations of ergosterol and para-

thyroid hormone there were found, depending on the duration of experiments, both primary and secondary injection stripes, and the fact that these stripes were associated with a rise and fall of the blood calcium respectively, (Schour and Ham ²) suggest a similar association in the experiments of this report. It is therefore possible that in each case there is at first a more or less sharp rise in blood calcium which results in the primary hypocalcified stripe, and subsequently a decline which results in the secondary hypercalcified stripe. It is also possible on the basis of Pugsley's work ¹⁵ that with 25 units of injections per day an increased excretion of calcium rather than a rise in blood calcium may be responsible for the presence of the primary hypocalcified stripe.

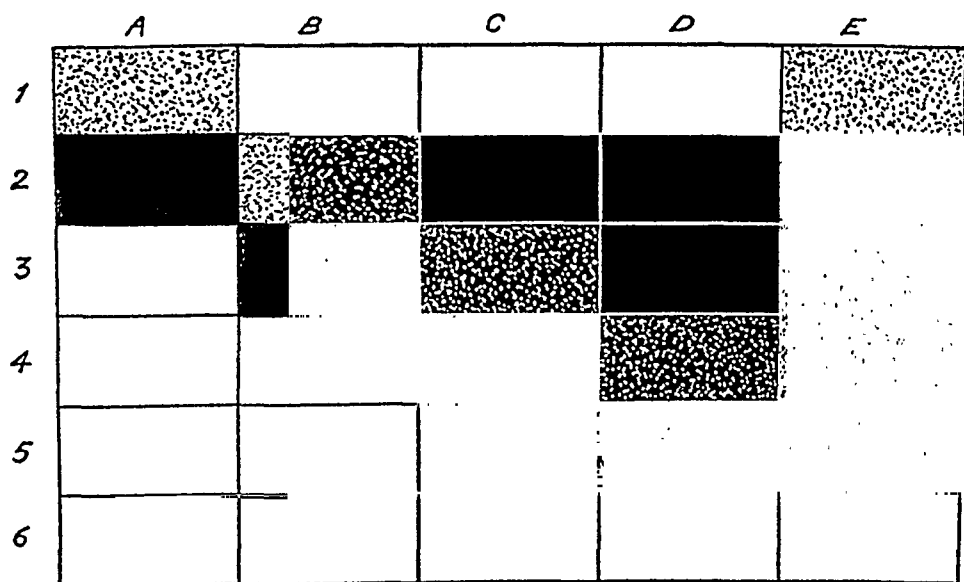
The association of a primary hypocalcified dentin stripe with hypercalcemia and of a secondary hypercalcified stripe with the return to a normal blood calcium value may be explained tentatively on the theory discussed in greater detail by Schour and Ham ⁸ that the parathyroid hormone controls a fraction of the serum calcium. Thus, an injection of the hormone results first in a shift of calcium from the tissues to the blood, possibly, to the fraction of the serum calcium controlled by the hormone either by calcium withdrawal or by the attraction of the calcium that is normally available for calcification, or both. With our present state of knowledge we are not in a position to state whether the hypocalcified condition of the primary dentin stripe was due to a calcium withdrawal or due to the fact that an insufficient amount of calcium was available when it was being calcified.

When the effect of the parathyroid hormone has passed the calcium that was held in solution under its influence is liberated and there results, therefore, a shift of calcium from the blood back to the tissues. Thus in the dentin that is being calcified at this time we find the secondary hypercalcified stripe, because a greater than normal amount of calcium is now available. It is also possible, according to Schour and Ham, ⁵ that the liberation of calcium from the blood might introduce more calcium into simple solution than could be retained without precipitation.

If in case of "multiple injection" experiments the injections were made at intervals sufficiently far apart so that the successive administrations were made only after the effect of the preceding ones had worn off, then we would have simply a series of pairs of primary

and secondary stripes, the number of pairs corresponding with the number of injections. We have a suggestion of such a reaction in Rat 306 (Fig. 13).

In the experiments in which the multiple injections were in daily succession only the primary stripe associated with the first injection remains relatively unchanged, while the rest of the stripes overlap and fuse into an extended secondary stripe which represents the summation of the effects of the successive injections (Figs. 6 and 8). It is likely that the blood calcium curve in such animals would show



TEXT-FIGURE 1

Diagram showing the histological effects of parathyroid hormone injections on the dentin. The horizontal sections represent the portions of dentin laid down in successive days, 1-6. The vertical columns, A-E, represent sections of dentin belonging to animals A-E respectively.

subsequent to the early rise a decline that was retarded by a plateau. In some of the experiments of multiple injections the histological findings suggest the presence of interruptions of the fall of blood calcium in the form of secondary rises associated with the repeated injections.

Text-figure 1 illustrates in diagrammatic form the histological effect of parathyroid hormone injections on the dentin. The findings in this report and that of Schour and Ham² indicate that if Rat A were given a single injection of parathyroid hormone at the beginning of the first day, Stripe 1 would be poorly calcified and Stripe 2 overcalcified, as indicated by column A, and shown in Text-figure 1;

similarly, if Rats B, C and D were given single doses on the second, third and fourth day respectively, the effects would be those indicated in columns B, C and D. It is obvious that if the above 4 injections were given on successive days to 1 Rat E instead of singly to 4, the effect would be that indicated in column E. The latter corresponds closely to the pattern seen in Figure 8 taken from a rat that was given 4 successive daily injections.

Blood Calcium Findings: Our blood calcium findings are of limited value because they indicate the condition immediately preceding death and therefore reveal only one point in the blood calcium curve of the experimental period. However, a comparison of the findings of animals that were given single injections and killed 19 hours later shows that the blood calcium rose in 4 of the 5 animals (Table I). Similarly a group of animals that were given 3 successive daily injections of 50 units each showed a prominent rise in blood calcium 19 hours after the last injection, while another animal with the same number and dose of injections, but distributed over a period of 10 days, indicated a return to normal at the time immediately before death.

Dentin Reaction in the Molar: The dentin in the molars appears to be normal in the majority of the experimental animals. In a few instances, however, the findings reveal an interesting picture. Figure 14 shows a prominent eosin-staining stripe, which is followed by a well calcified stripe that is next to an abnormally wide predentin border. The width of the region involved is approximately one-fourth that of the dentin region found to be disturbed in the incisor. If the molar dentin grew one-fourth the rate of the incisor dentin, one could assume that fundamentally the dentin reacts similarly in the incisor and molar. If the molar dentin grew much slower than one-fourth the rate of the incisor dentin one could assume the possibility of calcium withdrawal. The final explanation of the molar findings awaits further investigation.

Alveolar Bone in the Rat Incisor: Among the 21 rats that had 3 or more injections 14 showed an abnormally increased number of osteoclasts. The 7 rats that showed a normal alveolar bone include 2 that were given inactivated hormone and 2 that were given repeated doses of 10 units each. In 3 of the rats the alveolar bone shows a typical picture of osteitis fibrosa, in respect to the predominance of the osteoclasts and the fibrous change in the bone marrow (Fig. 11).

This is not at all surprising since the alveolar bone is highly active, especially at the age of the experimental animals. At this time the curvature of the incisor becomes flattened and the alveolar bone undergoes considerable adjustment to this change in contour. Bones that are most active have been found to be most susceptible to characteristic changes of experimental hyperparathyroidism (Jaffe ¹⁶).

Differences in the Reaction of Dentin and Alveolar Bone to Experimental Hyperparathyroidism: Dentin records delicate changes that are not recognizable in bone and tolerates insults in calcium metabolism that produce severe disturbances in bone. Figure 13 shows the dentin disturbance in Rat 306 which was given 14 injections of parathyroid hormone. Each injection is recorded. The last 3 injections, which were given 48 hours apart, produced wider secondary hypercalcified stripes than did the earlier injections given 24 hours apart. But on the whole, the dentin is well calcified in spite of the disturbance in rhythm and is capable of normal function. On the other hand, the alveolar bone changes in the same rat, shown in Figure 11, are much more severe. The osteoblasts are replaced by osteoclasts. The bone is resorbed and liable to fracture. The bone marrow is fibrous. It appears that, in confirmation of Erdheim's calcioprotective law,¹⁷ dentin possesses a greater tolerance against calcification disturbance than does bone.

On first glance there is an apparent contradiction between the high sensitivity and the high tolerance of dentin to changes in calcium metabolism. However, a consideration of the histophysiological processes of dentin formation and calcification shows that a given layer of dentin is found to be adjacent to a very rich blood supply only temporarily when it is being laid down in its organic form and when it is in the process of becoming calcified. At this stage it is very sensitive to changes in calcium metabolism. As new layers of dentin are laid down and the tooth is erupting forward a given calcified layer moves farther away from the blood supply and in its entirely avascular condition becomes less sensitive and more tolerant to disturbances in calcium metabolism. Moreover, the normal and slow process of secondary calcification may result in a recovery and improved calcification of a former primary hypocalcified stripe, seen in Figure 7.

The alveolar bone, on the other hand, while it is, as a rule, at no time exposed to as rich a blood supply as is found adjacent to the

newly formed dentin, is constantly penetrated by blood vessels and is in close proximity to them. It is, therefore, always exposed to changes in calcium metabolism disturbances. These considerations apply in a similar manner to other bones as well as to alveolar bone.

Variations in Calcification within the Same Incisor: The variations in calcification seen within the same tooth at various levels (Figs. 6, 7, 8 and 9) may be in part explained by corresponding variations in the proximity of blood supply at various levels of the tooth.

The blood supply of the pulp is richest in the posterior region and very poor in the anterior region. As a given stripe of dentin moves forward its posterior portion lies within the vicinity of a rich blood supply for a longer period than its anterior portion and is, therefore, subject to secondary calcification for a longer time. The anterior portion lies at the very outset within the vicinity of a poorer blood supply and moves farther away from it with the eruption of the tooth. Thus, the primary undercalcified stripe loses its prominence in its posterior portion (Fig. 7), and similar to the fate of the transplantation stripe of Erdheim¹ often shows greatest disturbance in the anterior zone (Fig. 9). There are, however, prominent variations seen within limited regions of the tooth which cannot be explained solely on the basis of blood supply. It seems that in the pathological disturbances induced in the experiments of this investigation the typical response itself suffers aberrations for reasons not yet known.

Sensitivity of Dentin: The dentin reaction in the guinea pig incisor has been employed successfully as an indicator for vitamin C deficiency. The delicacy of the dentin reaction to the various experimental conditions described in this report suggests the feasibility of using the dentin of the rat incisor as an indicator for a biological assay of the parathyroid hormone. The present common method of standardization of the parathyroid hormone by means of the blood calcium reaction in the dog is laborious and expensive. The authors believe that a simplified method would facilitate research on the parathyroid hormone. It is likely that further experimentation will obviate the necessity of histological preparation and evolve a simple method of preparing ground sections of the rat incisor which can be stained with alizarin or which can be obtained from rats given alizarin intravitaly.

SUMMARY AND CONCLUSIONS

The effect of single and multiple injections of parathyroid hormone on the incisor of the white rat has been studied in 29 animals and 7 controls in respect to microscopic alterations and blood calcium changes.

The histological findings are: (1) enamel hypoplasia formation begins immediately after the time of the first injection in 5 animals; (2) the alveolar bone shows an abnormal increase in osteoclasts in 14 and a fibrous change of the bone marrow in 3 of the rats that were given 3 or more injections of parathyroid hormone; and (3) the principal changes are found in the calcification of the dentin. The experimental animals show a primary hypocalcified stripe in the dentin that was being calcified during the immediate effect of the first injection and a secondary hypercalcified stripe in the dentin that was being calcified subsequently. The extent of and the variations within the secondary stripe vary with the number and unitage of the hormone preparations injected, and also with the duration of the experiment.

It is believed that the experimental pattern in the dentin represents its response to the changes in the calcium and phosphorous metabolism induced by the injections of the parathyroid hormone. The dentin reaction can be explained best by the theory that the parathyroid hormone controls a fraction of the serum calcium.

NOTE: We wish to thank Eli Lilly and Company for the parathormone used in these studies, and Swift and Company for their coöperation. We also wish to thank Miss F. Schwab and Mr. H. C. Breuhaus for their assistance in the preparation of the material.

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DESCRIPTION OF PLATES

PLATE 89

- FIG. 1. Photomicrograph of longitudinal section of midregion of upper incisor of a normal rat. Note the predentin layer, P, which stains neither with hematoxylin nor eosin. The eosin-staining late predentin is absent. D = normal calcified dentin; OD = odontoblasts. $\times 455$.
- FIG. 2. Photomicrograph of longitudinal section of midregion of upper incisor of Rat 317, which received one injection of 75 units of parathyroid hormone and was killed 19 hours later. Note the irregular boundary between the early, E, and late predentin, L, and the scattered eosin-staining globules in the early predentin. The secondary hypercalcified stripe is absent because the animal was killed too soon. D = dentin which is normal and which was calcified before the experiment began; OD = odontoblasts. $\times 427$.
- FIG. 3. Photomicrograph of longitudinal section of midregion of upper incisor of Rat 318 which received 1 injection of 100 units of parathyroid hormone. Note the poor, incomplete transition of early, E, into late predentin, L, and the scattered eosin-staining globules, GL, in the early predentin. Note the absence of the secondary hypercalcified stripe. OD = odontoblasts. $\times 427$.
- FIG. 4. Photomicrograph of transverse section of lower incisor of Rat 337, which was given 3 injections of 25 units each of parathyroid hormone. Both the primary hypocalcified stripe, PR, and the secondary hypercalcified, SE, are not prominent. The total width of both stripes corresponds with the amount of dentin that is laid down during the time of the duration of the experiment. Compare the staining reaction of the dentin formed before, D, and after the injections, PR and SE. OD = odontoblasts; PU = pulp. $\times 101.5$.
- FIG. 5. Photomicrograph of longitudinal section of midregion of upper incisor of Rat 374, which was given 1 injection of 150 units of parathyroid hormone and was allowed to live 48 hours. Note the primary hypocalcified, PR, and the secondary hypercalcified, SE, stripes. D = dentin calcified before the experiment began; OD = odontoblasts. $\times 210$.
- FIG. 6. Photomicrograph of longitudinal section of posterior and midregion of upper incisor of Rat 301, which was given 4 injections of parathyroid hormone and was allowed to live 3 days after the last injection. Note the enamel hypoplasia, "x," at point where the primary hypocalcified stripe, PR, begins. Note the deep stain of the secondary hypercalcified stripe, SE. D = normal calcified dentin; EP = enamel epithelium; ES = enamel space; OD = odontoblasts; OE = organic enamel matrix; PU = pulp. $\times 2.24$.
- FIG. 7. Photomicrograph of area indicated in Fig. 6 and magnified. Note the partial obliteration of the primary hypocalcified stripe, PR, and compare

with the same stripe in Fig. 8. D = normal calcified dentin; OD = odontoblasts; OE = organic enamel; P = predentin; PU = pulp; SE = secondary hypercalcified stripe. $\times 210$.

FIG. 8. Photomicrograph of area indicated in Fig. 6 and magnified. Note the prominent primary hypocalcified, PR, and secondary hypercalcified, SE, stripes in the dentin. Note the dentin, D, that was calcified before the experiment began. The width of the dentin ($90\ \mu$) laid down during the experiment approximates the amount of dentin that would be laid down normally in 6 days, the duration of the experiment. OD = odontoblasts; P = predentin. $\times 210$.

FIG. 9. Photomicrograph of region near the anterior end of the pulp of upper incisor of Rat 301, which was given 4 injections of parathyroid hormone. Note the primary injection stripe, PR, the secondary injection stripe, SE, which is interrupted by an eosin-staining stripe and severely disturbed in its portion toward the pulp, as evidenced by the predentin, P, which is abnormal in width, irregular in its course and contains vascular inclusions. Compare with Figs. 6, 7 and 8 taken from the same histological preparation. D = normal calcified dentin; OD = odontoblasts; PU = pulp. $\times 210$.

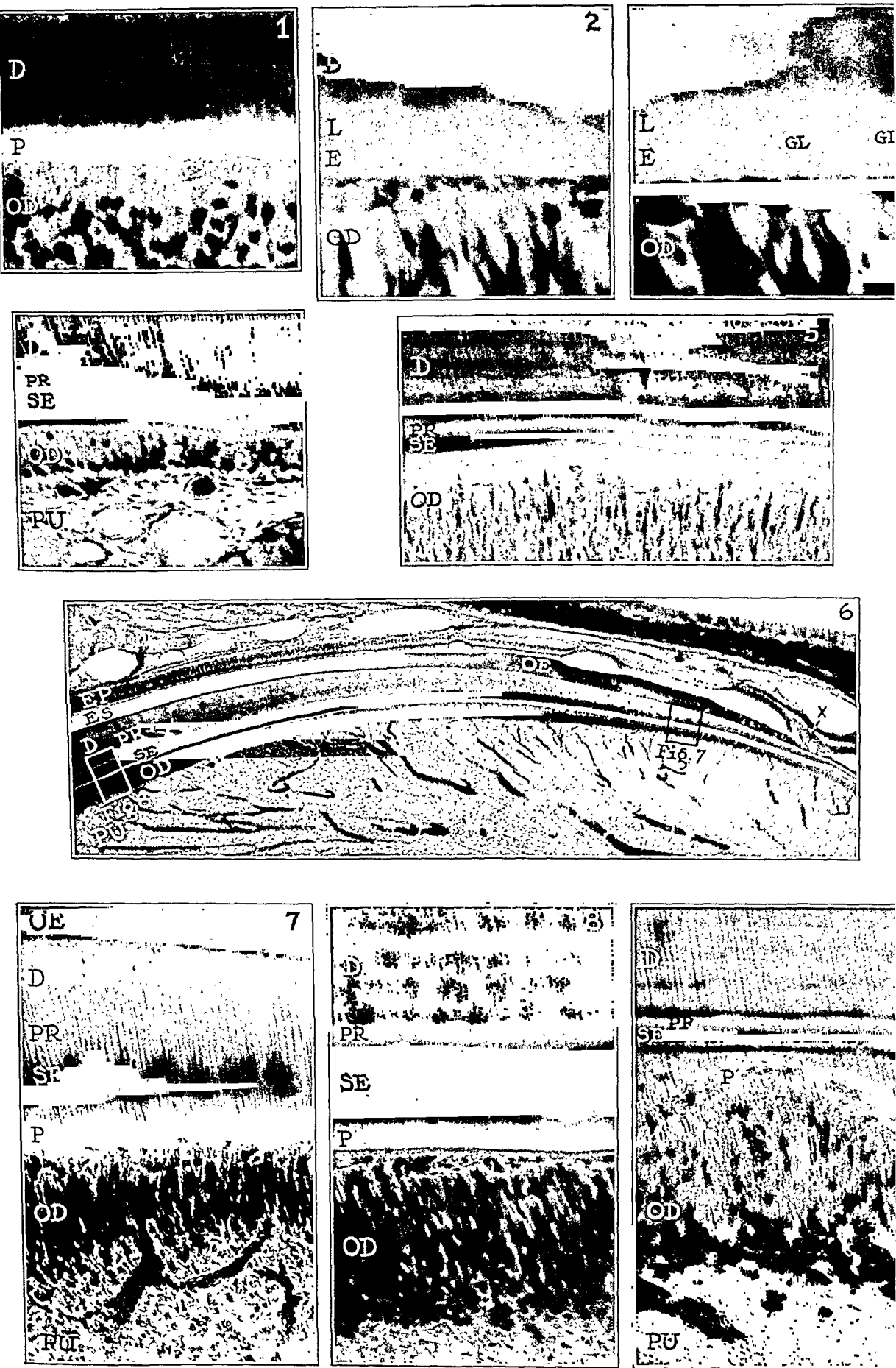


PLATE 60

FIG. 12. Photomicrograph of section near the lingual alveolar crest of upper incisor of control Rat 350. Note the rich number of osteoblasts, OB, lining the bone marrow space, BM. Compare with Fig. 11. $\times 101.5$.

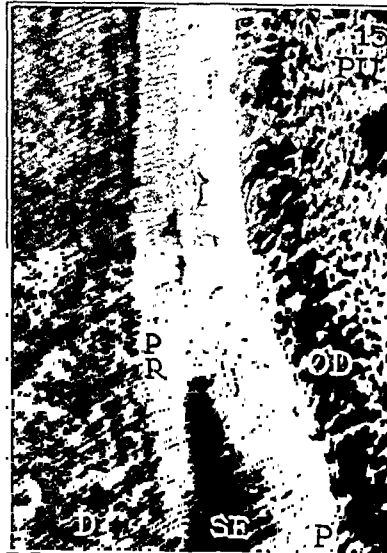
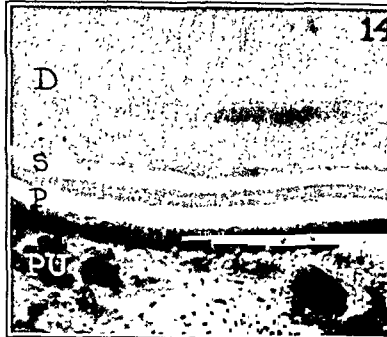
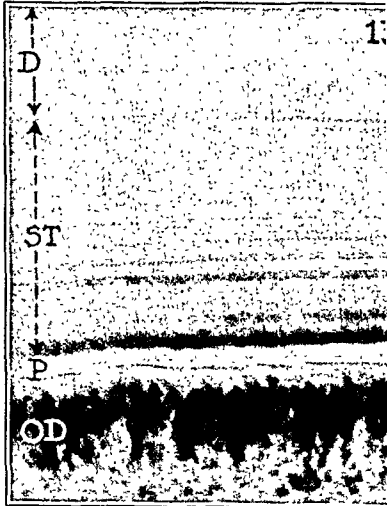
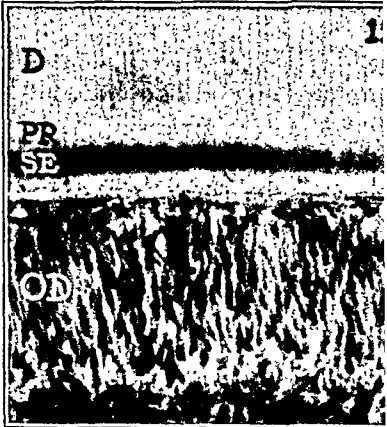
FIG. 13. Photomicrograph of section near the lingual alveolar crest of upper incisor of Rat 359, which received 12 injections of parathyroid hormone. Note the absence of osteoblasts; the fibrous change of the bone marrow, BM; the presence of a number of osteoclasts, OC. Compare with Figs. 10 and 11. $\times 101.5$.

FIG. 14. Photomicrograph of a longitudinal section of midregion of upper incisor of Rat 352, which was given 4 injections of parathyroid hormone and was killed 10 hours after the last injection. Note the primary, PR, and secondary, SL, injection stripes. D = dentin which is normal and which was calcified before the experiment began; OD = odontoblasts. $\times 106$.

FIG. 15. Photomicrograph of dentin of midsection of incisor of Rat 307, which received 12 injections of parathyroid hormone. Note the stratification, SL, which is prominent in the portion of the dentin extending over 250 μ , which approximates the amount of dentin that is laid down normally in 18 days. Compare with the pre-experimental dentin, D. OD = odontoblasts; P = predentin. $\times 105$.

FIG. 16. Photomicrograph of the dentin of the floor of the pulp chamber of the first lower molar of Rat 323, which received 6 injections of 50 units each of parathyroid hormone. Note the eosin-staining dentin stripes, S, situated within the dentin, D, and the abnormally wide predentin border, P, next to the pulp. PU = pulp.

FIG. 17. Photomicrograph of transverse section of lower incisor of Rat 324, which received 6 injections of 50 units of parathyroid hormone. Note the eosin-staining dentin stripes, PR, and the disturbance or interruption of the secondary stripe, SL, which at the top of the field is poorly calcified and contains cellular inclusions. D = dentin calcified before the experiment; OD = odontoblasts; PU = pulp. $\times 100$.



MICROGLIA-LIKE CELLS AND THEIR REACTION FOLLOWING INJURY TO THE LIVER, SPLEEN AND KIDNEY *

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Certain cells in the nervous system which had hitherto been difficult to stain were included by Cajal¹ in 1913 under the term "third element" of the central nervous system, and were suspected by him to be of mesodermal origin. Somewhat later del Río-Hortega²⁻⁵ was able to show that this "third element" consisted of two entirely different kinds of cells: one kind was the oligodendroglia of ectodermal origin; and the other kind he called microglia, the latter, in his opinion, being of mesodermal origin. The central nervous system was made up, therefore, of neurones (the first element), neuroglia consisting of astrocytes and oligodendroglia (the second element), and microglia (the third element). Del Río-Hortega's papers describing the microglia were published between 1919 and 1921. Today del Río-Hortega⁶ believes, along with others, that the microglia represents the reticulo-endothelial system in the central nervous system.

In 1921 del Río-Hortega and Jiménez de Asúa⁷ demonstrated phagocytic cells in tumors, tubercles, liver lesions, normal human kidney, and in lymph follicles stained by silver carbonate. In this paper the authors drew attention to their belief that phagocytosis in the nervous system is the function of the microglia and that the studies of del Río-Hortega on the microglia constitute a concrete example of the general problem of the histogenesis of the macrophages.

In 1927 Jiménez de Asúa,⁸ using silver carbonate, demonstrated macrophages in a normal spleen and in tumors, and stated that in morphology, staining properties and in function they resemble the microglia of the nervous system and, finally, that microglia cells are members of the reticulo-endothelial system.

Cone,⁹ in 1928, using del Río-Hortega's method for microglia, illustrated a phagocyte with a close resemblance to transitional microglia in a degenerating area of a hypernephroma.

* Received for publication March 1, 1934.

Dorothy Russell¹⁰ in 1929 demonstrated intravital staining of microglia with trypan blue and concluded that such intravital staining identifies microglia with the rest of the reticulo-endothelial system and that microglia is a mesodermal element.

Wells and Carmichael¹¹ in 1930 made a study of microglia by means of tissue culture and vital staining and concluded that microglia is of mesodermal origin and is analogous to the resting histiocytes or fixed macrophages of the reticulo-endothelial system. They also found microglia-like cells in cultures from embryonic chick periosteum and limb-bud and found that these cells reacted to vital dyes *in vitro* in the same manner as wandering cells.

Visintini¹² in 1931 demonstrated microglia-like cells in the heart, voluntary muscle and urinary bladder by the method of Bolsi.

Belezky¹³ in 1931, using silver carbonate, demonstrated cells in the spleen which he regarded as reticulo-endothelial cells. These cells, in our opinion, resemble microglia cells.

The microglia cells appear in the nervous system at about the time of birth. They migrate to all parts of the brain and spinal cord and remain there inactive until the advent of some disease. When this occurs the first pathological change that can be noticed in certain types of lesions is the sudden and tremendous activity of the microglia. They multiply, migrate in great numbers to the damaged area and become phagocytic, devouring the broken-down nervous tissue and other debris and apparently removing it to the nearest perivascular spaces, where they appear in the form of large, rounded, compound granular corpuscles loaded with fat (*Gitterzellen*). Thus they clear the way for the astrocytes to lay down a scar in place of the destroyed tissue.

A similar phenomenon might occur in other tissues of the body. With this possibility in mind experiments were performed on rabbits, using del Río-Hortega's original silver carbonate method of specific staining for microglia in the liver, spleen and kidney after producing a destructive lesion in these organs.

Under sterile conditions a cerebral hemisphere, the liver and the spleen of a rabbit were punctured by a hot trocar, ether anaesthesia being used. At the end of 4 days the animal was killed and the brain, liver and spleen were fixed in Cajal's formal-bromide solution for about 24 hr. Portions were cut from the traumatized portions of each of the three organs and stained at the same time by

del Río-Hortega's original silver carbonate method for microglia. In Figure 1 the area of brain destroyed by the hot trochar is surrounded by great numbers of microglia cells, forming a dark ring about the necrotic area. Figure 2 is a high power view of a portion of this ring. The microglia cells can be seen in different stages of metamorphosis from the almost normal cell with its spiked processes to the completely formed compound granular corpuscle loaded with fat. Figure 3 is a low power photomicrograph of a portion of the lesion in the liver. The margin of the necrotic area is marked by a dark ring of cells which in the high power view (Fig. 4) closely resemble the transitional microglia of the brain. These cells contain droplets of fat. Cells of the same character are present at the margin of the puncture wound of the spleen. The stages in the transformation of a cell with spiked processes into a swollen, rounded form are illustrated in Figures 5 to 10 by camera lucida drawings of six cells found at the margin of the necrotic area in the same spleen. All of the cells except the first contain droplets of fat, which appear black in the picture. We wish to draw attention to the close resemblance of the cells in this figure to the microglia cells illustrated in Plate 91 of Dorothy Russell's article, referred to above.

Using the same technique as in the first experiment a kidney of a rabbit was punctured by a hot trochar. The animal was killed at the end of 4 days and the injured organ was fixed in formol-bromide. Sections of the lesion were cut and stained in the same manner as were the brain, liver and spleen. Figure 14 is a high power view of fat-containing microglia-like cells in the process of swelling at the margin of the damaged area in the kidney. Some of these cells are elongated and have small spikes, very much like the microglia cells in a paretic brain (the "rod-cells" or *Stäbchenzellen* of general paralysis).

A few hours before a puncture wound of the spleen was made 10 cc. of a 1 per cent aqueous solution of trypan blue were injected into an ear vein of a rabbit. The animal was allowed to live 4 days, and during this period 35 cc. of the same solution of trypan blue were injected intraperitoneally. The spleen was fixed and sections of the lesion were cut and stained as before, with the exception that the silver impregnation was not toned in gold chloride because it tended to change the blue dye to purple. Figure 11 is a low power view of the damaged area outlined by a well marked dark ring of cells.

In the high power view of a portion of the ring (Fig. 12) many swollen cells, which closely resemble pathological microglia, can be seen, and all of the cells in this picture contain trypan blue in granular form. Figure 13 is a high power photomicrograph of a group of cells in a splenic nodule at some distance from the lesion. These cells closely resemble slightly swollen microglia, and small spike-like projections on their main processes can be seen, a feature characteristic of the microglia of the nervous system. Trypan blue could not be demonstrated in this group of cells. By staining two consecutive sections of the block, one with silver carbonate and the other with hematoxylin and eosin, the opposite halves of the same cells impregnated with silver were colored by the organic dyes. Stained by the more familiar method the cells pictured in Figure 12 have the following characteristics. Compared with the nucleus of the lymphocyte the nucleus of the argyrophilic cell is larger, less deeply stained by hematoxylin, and varies in shape, tending to be rounded, oval or kidney-shaped. It contains a nucleolus and a uniform distribution of fine granules of chromatin. The clear cytoplasm is pale pink, almost colorless, and contains large and small granules of trypan blue, masses of amber blood pigment and an occasional engulfed lymphocyte. The shape of the cell is as varied as the shape of the nucleus and its processes, which are so clearly impregnated with silver, cannot be distinguished in the section stained with hematoxylin and eosin.

The next step was to determine whether microglia-like cells are present in the normal liver, spleen and kidney as they are found in a resting state in the normal nervous system and whether they could be demonstrated by the same technique of staining. Accordingly, the liver, spleen and kidneys of normal rabbits were stained by del Rio-Hortega's original silver carbonate method for microglia. Figure 14 is a high power photomicrograph of the normal liver of a rabbit. In the center of the field there is a triangular-shaped cell with three long processes extending between the liver cells. Typical of many others scattered throughout the liver and undoubtedly representing the nearly normal or very early transitional form of the cells demonstrated in Figures 3 and 4, it is morphologically similar to the macrophage cells of the nervous system. In Figure 15, a high power photomicrograph of the normal spleen of a rabbit, one slightly swollen microglia-like cell is seen at the edge of a splenic nodule.

This cell is typical of many others found at the periphery of the nodules and these undoubtedly are the source of such cells as those demonstrated in Figures 5 to 10, 11 and 12. Figures 17 and 18 are high power photomicrographs of microglia-like cells in a normal kidney of a rabbit. Spike-like projections on their processes are well shown. Many similar cells were found scattered between the tubules throughout the kidney and they are undoubtedly the resting or early transitional forms of the cells demonstrated in Figure 14. In the normal organs that were studied all of the microglia-like cells were somewhat swollen, as if they were constantly being stimulated to activity.

SUMMARY

1. Cells have been demonstrated by del Río-Hortega's original silver carbonate method of specific staining for microglia in the liver, spleen and kidney of the rabbit that in morphology are identical with the nearly normal or very early transitional forms of microglia in the nervous system.
2. In their reaction to injury and to the intravital injection of trypan blue they have been shown to be identical with microglia.
3. These cells have been demonstrated in a transitional stage with spiked processes like microglia and containing droplets of fat or granules of trypan blue.
4. By the silver carbonate method of staining earlier transitional forms have been demonstrated that contain no visible amounts of fat or trypan blue.
5. A more advanced transitional form has been shown in preparations of the spleen of the rabbit to be a histiocyte or large mononuclear phagocyte without processes and containing droplets of fat, granules of trypan blue, blood pigment and engulfed lymphocytes.

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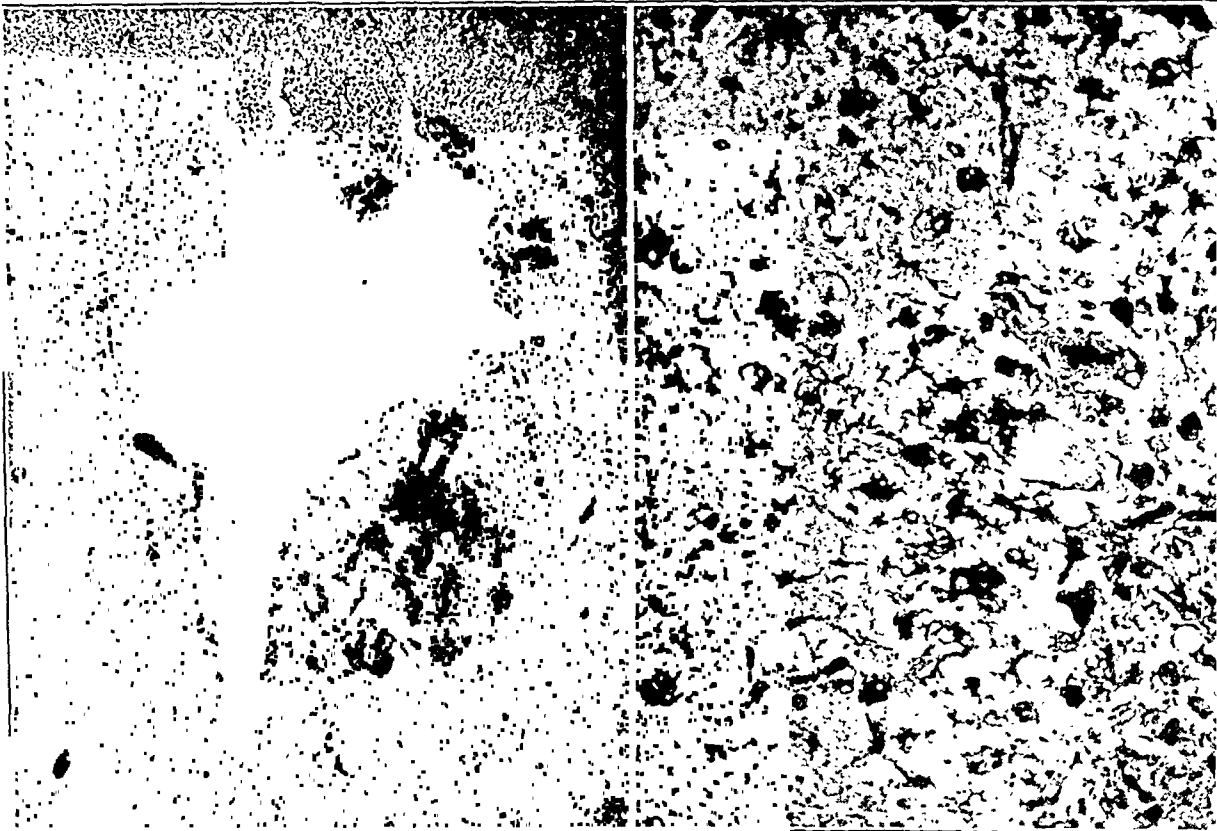
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DESCRIPTION OF PLATES

PLATE 91

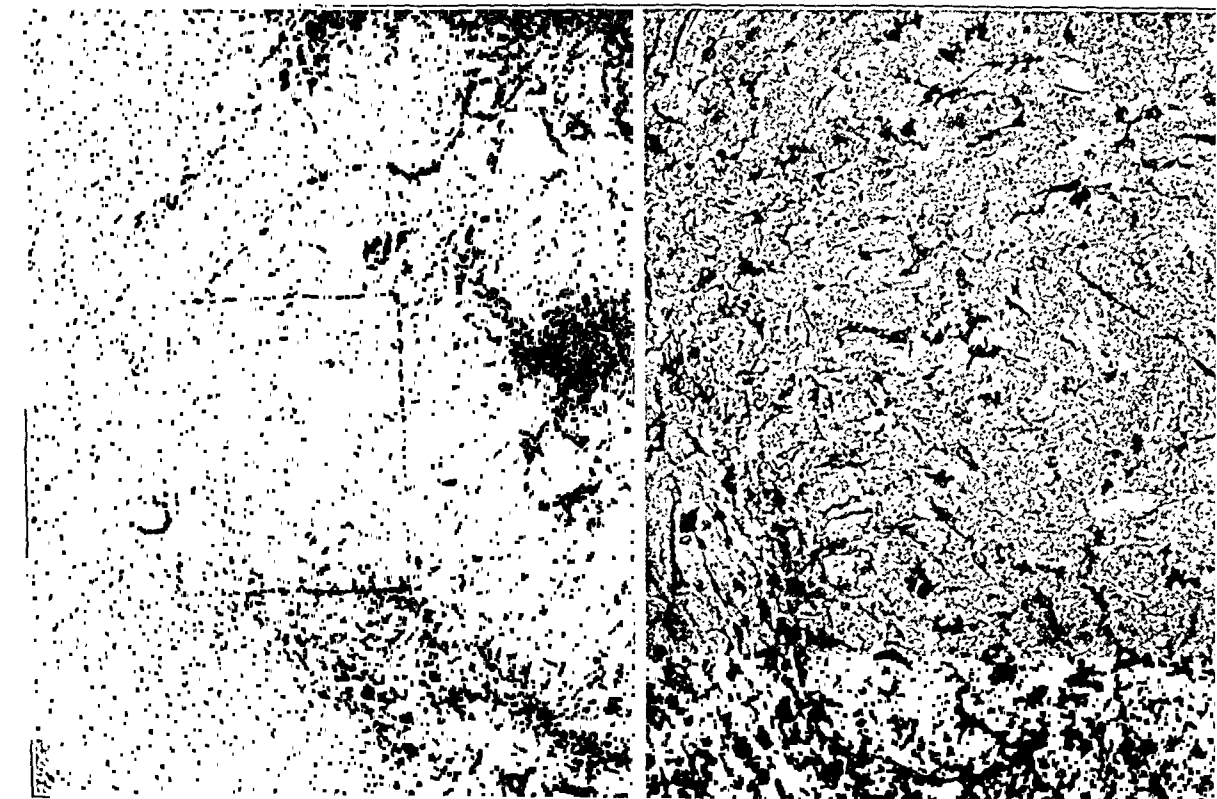
We are indebted to Mr. William S. Dunn for the photomicrographs illustrating this paper.

- FIG. 1. Microglia cells in the cerebrum of a rabbit forming a dark ring about a receptive lesion produced by puncture with a hot toothpick. Silver carbonate stain for microglia. $\times 21$.
- FIG. 2. A high power view of cells in the area of Fig. 1 outlined in black, showing the transformation of cells with spined processes into rounded forms filled with droplets of fat. Silver carbonate stain for microglia. $\times 106$.
- FIG. 3. A portion of a receptive lesion in the liver of a rabbit produced by puncture with a hot toothpick. Microglia-like cells are gathered at the margin of the lesion and are stained for microglia. $\times 46$.
- FIG. 4. A high power view of cells in the area of Fig. 3 outlined in black, showing the transformation of cells with spined processes into rounded forms filled with droplets of fat. Silver carbonate stain for microglia. $\times 106$.



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PLATE 92

FIGS. 5-10. Camera lucida drawings of six cells found at the margin of a necrotic area produced by a hot trocar in the spleen of a rabbit, illustrating the stages in the transformation of a microglia-like cell with spiked processes into a large rounded form. Note the apparent transition of the processes into rounded projections resembling pseudopodia. All of the cells except the first contain droplets of fat, which appear black in the picture. Silver carmalum stain for microglia with Sudan III. $\times 950$.

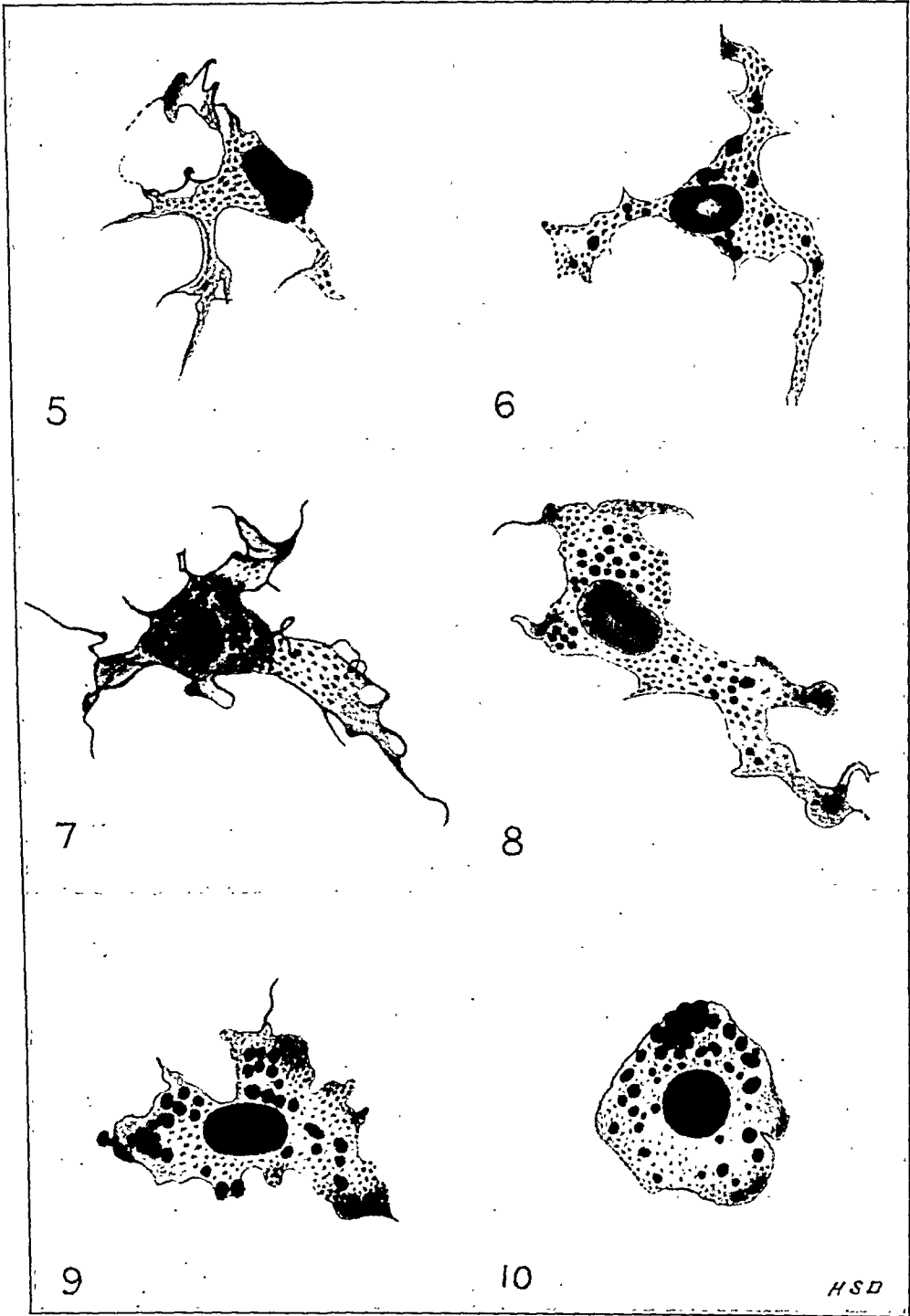
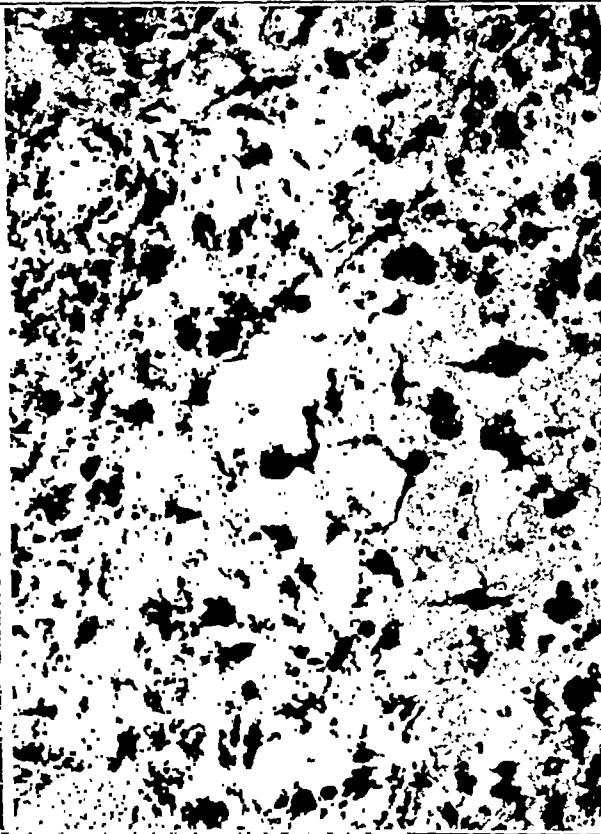


PLATE 63

- FIG. 11. A necrotic lesion outlined by a dark ring of cells produced by a hot trocar in the spleen of a rabbit injected intravitaly with trypan blue. Intravital trypan blue and silver carbonate stain for microglia. $\times 126$.
- FIG. 12. A high power view of cells at the margin of the lesion in Fig. 11 in the area outlined in black. Granules of trypan blue are present in microglia-like cells with spiked processes and in the larger rounded forms. Intravital trypan blue and silver carbonate stain for microglia. $\times 140.24$.
- FIG. 13. Cells resembling slightly swollen microglia in a splenic nodule at a distance from the lesion in the spleen pictured in Fig. 11. Note the numerous spikes on their main processes. Trypan blue could not be demonstrated in this group of cells. Intravital trypan blue and silver carbonate stain for microglia. $\times 120.24$.
- FIG. 14. A high power view of fat-containing microglia-like cells in the kidney of a rabbit gathered at the margin of a necrotic lesion produced by a hot trocar. Note the elongated forms with spikes resembling the rod cells in a parietic brain. Silver carbonate stain for microglia. $\times 126$.



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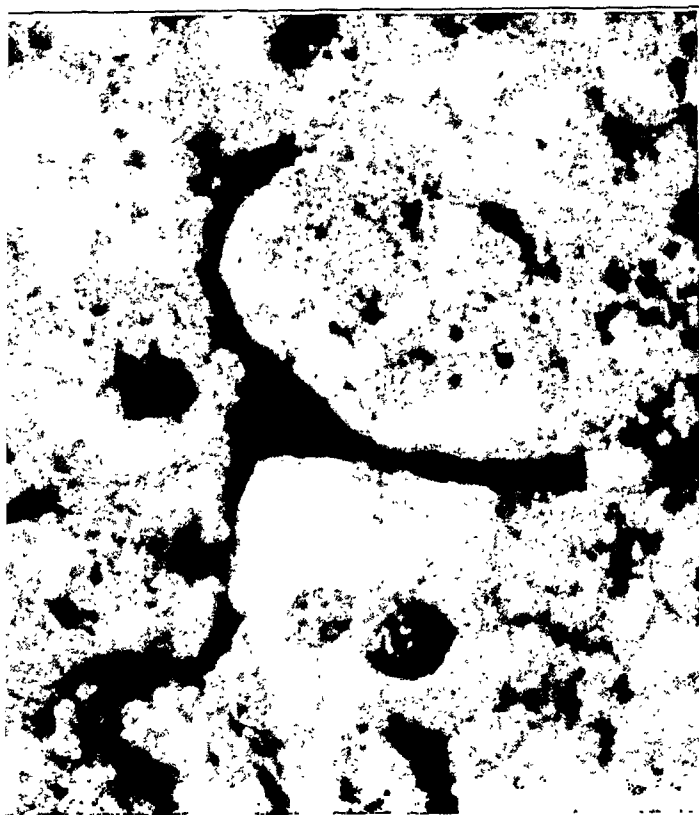
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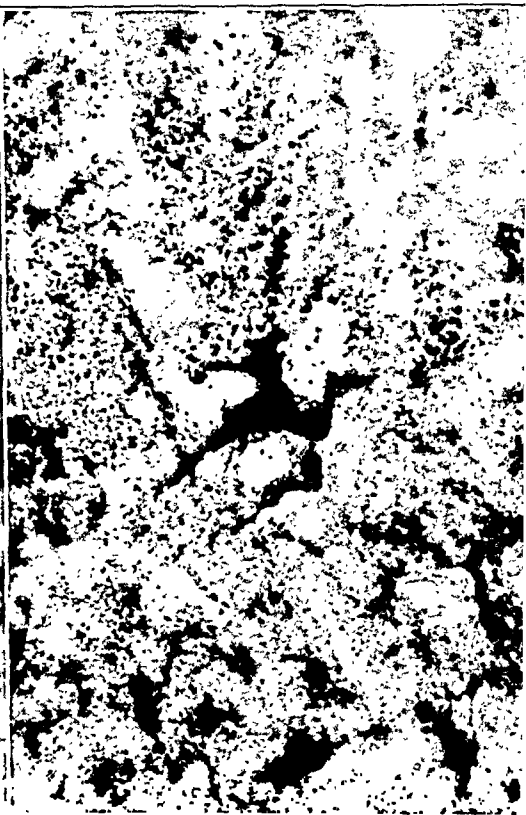
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PLATE 64

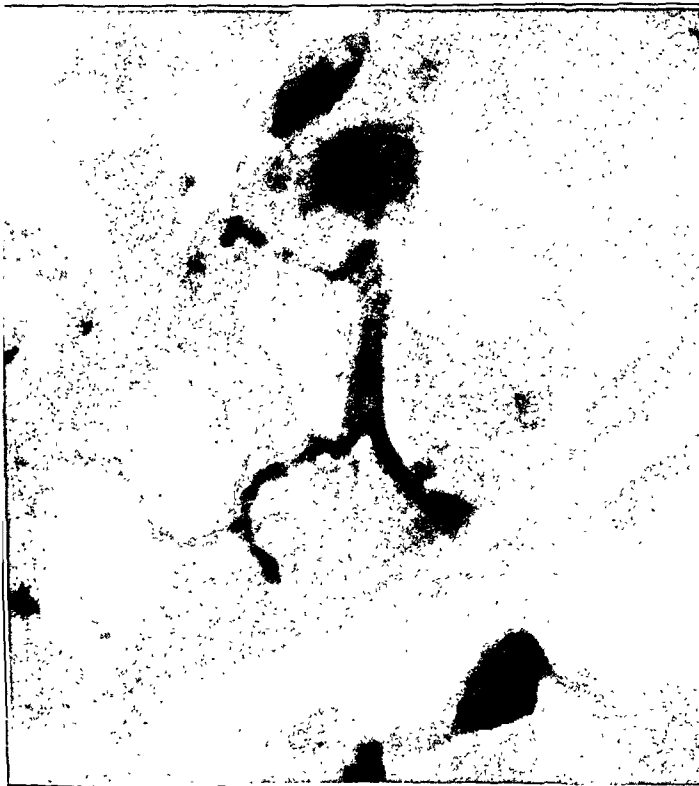
- FIG. 15. A cell in the liver of a normal rabbit resembling a nearly normal or very early transitional microglia cell. Note the manner in which its three processes insert themselves between the liver cells. Silver carbonate stain for microglia. $\times 950$.
- FIG. 16. A cell resembling a slightly swollen microglia cell at the edge of a splenic nodule in the spleen of a normal rabbit. Silver carbonate stain for microglia. $\times 650$.
- FIG. 17. A cell resembling an early transitional microglia cell in the kidney of a normal rabbit. Silver carbonate stain for microglia. $\times 1200$.
- FIG. 18. Another cell in the kidney pictured in Fig. 17 resembling a nearly normal or very early transitional microglia cell. Note the spikes on its main process and its position between the kidney tubules. Silver carbonate stain for microglia. $\times 950$.



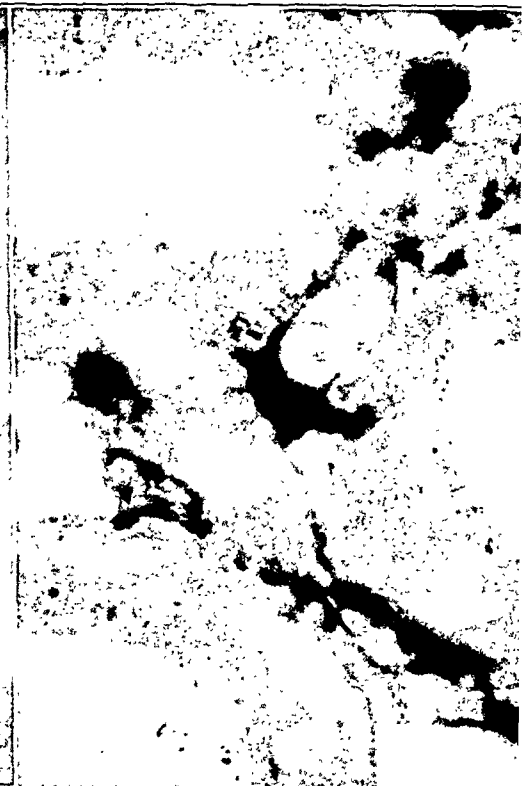
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POLYARTERITIS NODOSA *

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NOMENCLATURE

In 1865 Kussmaul and Maier¹ described an arteritis characterized by the formation of multiple circumscribed nodulations in the small arteries of various parts of the body. They named it periarteritis nodosa, a term that implies inflammatory changes in and around the adventitia of arteries with the production of nodules, and one that was intended as a précis of the morphological peculiarities of the condition. We now know that the inflammation is by no means confined to the adventitia, that the primary changes are probably in the media, and that the disease usually affects a large number of vessels. In recognition of these facts Dickson² suggested the name polyarteritis nodosa, which is a more accurate epitome of what we know of the condition, and which seems to us preferable to the older term.

INCIDENCE

Approximately 150 cases of polyarteritis nodosa have been reported, less than 20 of them in the American literature (although in this country a number of excellent summaries have appeared, notably those of Ophüls,³ and Lamb⁴). The greater number of routine autopsies in European clinics partly explains the relative infrequency with which the disease is recognized in this country, for ante mortem diagnosis is made in not more than 20 per cent of cases. However, the lesion is rare even when histological studies are made in a large series of autopsies. In 2035 autopsies at the Peter Bent Brigham Hospital Bennett and Levine⁵ found only 2 cases. At the Los Angeles County Hospital, during the past 15 years (1918-1933), there have been 10,000 autopsies, only 1 of which showed the classical pathological findings of polyarteritis nodosa.

* Received for publication October 17, 1933.

It is conceivable, of course, that there may be a mild or early form of the disease in which the arterial changes are so slight as to escape detection in the routine microscopic study of autopsy sections. Clinically there can be no doubt that many cases have been completely unsuspected or misinterpreted, for polyarteritis nodosa habitually masquerades as one of the commoner infectious diseases. It is generally believed to be almost uniformly fatal, usually in the course of a few weeks, but the high mortality and the low incidence may both be explained partly by the fact that with few exceptions only the fatal cases have been recognized. Some authors believe that the total number of recorded instances grossly underestimates the actual frequency of the malady, and that many mild cases have undergone spontaneous recovery.

ETIOLOGY

Of the many propounded theories of etiology none has been generally accepted. For some time there was a tendency to regard the disease as a sort of aberrant manifestation of syphilis or some other systemic infection. The present tendency is to regard polyarteritis nodosa as a disease in its own right, quite independent of any underlying condition, and incrimination of syphilis seems utterly without foundation.

The febrile course and other outstanding symptoms are those commonly associated with sepsis and there is every reason to suspect an infectious origin. The small blood vessels in other infections, such as the cerebral vessels in influenza and the skin capillaries in epidemic meningitis, may show comparable lesions, and Bennett and Levine³ refer to the similarity between the pathological findings in polyarteritis nodosa and those of Rocky Mountain spotted fever and typhus fever, as discussed by Wollbach,⁴ and Wollbach, Todd and Paliney.⁵

Assuming that we are dealing with an infectious disease, we still have to decide whether there is a specific infective agent, or whether a variety of agents may produce the same striking vascular phenomena. Spiro⁶ does not consider polyarteritis nodosa a disease *in genere*; he thinks it merely one of the forms that may be taken by a monarteritis due to a variety of infections. Gruber^{7,8} states: "We regard periarthritis nodosa as the expression of a com-

stant characteristic reactive process of the arterial system in the manner of an hyperergic phenomenon during the course of very different infectious-toxic diseases. This is hypothesis!" However, when we contrast the enormous number of infections with the rarity of the histological phenomena seen in polyarteritis nodosa, it is difficult to believe that the vessels are capable of such a constant specific response to non-specific excitants.

Evidence is accumulating in favor of the specific infectious nature of the disease. Harris and Friedrichs,^{11, 12} though their experiments have not been widely accepted, believe they have produced the disease in animals. Other workers have observed in the lower animals (deer, calf, pig, dog) lesions almost identical with those we see in man. Altogether, the probabilities are that this peculiar and unmistakable lesion is induced by a specific infectious agent, probably a filterable virus with a predilection for the arterial system.

CLASSIFICATION

Obviously there can be no etiological classification. Histologically the lesions are readily grouped according to the stage of advancement of the inflammatory process. Clinically the victim's progress will depend upon the anatomical locations of the lesion and the severity of the arterial damage in these locations, so that we may attempt to divide polyarteritis nodosa into different types, according to predominant symptoms or the rapidity of its course. Sometimes the most battle-scarred areas may be mirrored in the clinical picture, but often the autopsy findings are the only reliable evidence upon which we may say that the disease is renal, cardiac, cerebral, abdominal, dermatological or neuromuscular in type.

PATHOLOGY

The affected vessels may often be recognized in the gross by the multiple small (peas-in-a-pod) nodules scattered along their course. These nodules may be inflammatory foci or actual aneurysms. Microscopically the periarterial connective tissue, the adventitia and the media show dense cellular infiltration consisting largely of polymorphonuclear leukocytes, and the media often shows extensive necrosis which may give rise to aneurysms. The intima shares the inflammatory process and, if the endothelium and internal elastic

lamina are destroyed, thrombosis results. Vascular occlusion with consequent infarction of the organs supplied by the affected vessels is the pathological key to the sudden critical clinical symptoms that may appear in the course of the disease. These vary according to the location and size of the occluded vessels.

The selection of the media rather than the adventitia as the primary site of attack (contrary to earlier conceptions) was demonstrated by Fishberg¹² in a patient who suffered from an acute form of the disease and died in a few days. The vessels affected are the small or medium sized muscular type arteries, while the elastic type escape. Arkin¹⁴ says that the organs most frequently involved are the kidneys (80 per cent), heart (70 per cent), liver (65 per cent), gastro-intestinal tract (50 per cent), pancreas (25 per cent), mesenteric artery (30 per cent), muscles (30 per cent), and peripheral nerves (20 per cent). The central nervous system is attacked in 8 per cent of the cases. The disease may confine itself to one organ for considerable periods of time, and involvement of other organs is irregular and wholly unpredictable. Sometimes the characteristic nodules appear in the skin, and a diagnosis is then readily made by excision of one of these lesions.

In his illuminating analysis of the pathological findings Arkin divides the disease into four stages: (1) the alterative-degenerative or beginning stage; (2) the acute exudative inflammatory stage; (3) the granulation tissue stage; and (4) the healed end-stage, or scar tissue stage. Of course, these stages are no more sharply separable than those of any other inflammatory process. They may merge and vary enormously in each individual case, and sometimes may telescope into one short fulminating bout in which all the stages seem to occur almost simultaneously with terrifying dispatch.

CLINICAL SYNDROME

The victim of polyarteritic nodosa is usually a man (males predominate four to one) between 20 and 50 years of age (the youngest reported case was 3 months, the oldest 78 years of age). The typical onset is said to be acute, with a short septic course ending in death often due to hemorrhage from a ruptured aneurysm in a few weeks. However, more often than most textbooks quote, the onset is gradual and insidious. The course is subtle, capricious and relatively pro-

longed. Arkin's case of histologically healed polyarteritis nodosa lived 4 years after his single attack of acute illness.

Evidences of sepsis are the rule — fever, high leukocytosis (and in our case eosinophilia), anemia, prostration and sometimes splenic tumor. Concomitant with these there are other more variable signs and symptoms that reflect the hidden vascular insults. The cardiac type behaves like a case of coronary sclerosis and there is apt to be an anginoid syndrome with manifestations of myocardial insufficiency. When the kidney is chiefly involved the course is often indistinguishable from essential hypertension with nephrosclerosis. There may be hypertension, visual disturbances and renal insufficiency, and if infarction occurs there is likely to be sudden hematuria. When the mesenteric vessels are occluded the symptoms may simulate those of an acute abdominal condition, and differential diagnosis is extremely difficult when the surgeon is confronted with a patient who has abdominal pain, fever, leukocytosis, nausea and vomiting. Neuromuscular symptoms of pain and tenderness along the peripheral nerves and in the muscles occur to some degree in most forms of the disease. It has been supposed that the neuritis was always secondary to changes in the arteries accompanying the nerves, but Carr's case¹⁵ seems to confirm the work of other investigators (*e.g.*, Wohlwill,¹⁶) who believe that there may be severe parenchymal nerve degeneration with or without arterial damage. Fletcher,¹⁷ Dickson,² and Bennett and Levine⁵ reported cases of the cerebral type of polyarteritis nodosa, and Bennett and Levine's second patient developed a meningitis, during the active stage of which there was an increased number of polymorphonuclear leukocytes in the cerebrospinal fluid, though no organisms could be detected.

DIFFERENTIAL DIAGNOSES

Small wonder that with such bizarre manifestations polyarteritis nodosa with monotonous regularity should pass wholly unsuspected (in about 88 per cent of cases probably) before autopsy. In our case, as in many others, even autopsy failed to reveal the condition until tissue sections were seen under the microscope.

During life the disease is usually mistaken for neuritis, myositis, trichinosis, vascular nephritis, typhoid fever, miliary tuberculosis, gastro-enteritis, pyemia, purpura hemorrhagica, hemorrhagic ne-

phritis, endocarditis, or acute abdominal conditions. It would be academic to point out features that might distinguish polyarteritis nodosa from each of these conditions. Indeed, it is seldom necessary or possible to make a careful differentiation. The diagnosis is usually unprovable but the possibility of a common vascular cause should be considered whenever a patient with sepsis exhibits a wide variety of symptoms, not peculiar to a specific disease. When an obscure sepsis cannot be fitted into one of the commoner infectious groups, the internist should consider polyarteritis nodosa as a possible diagnosis.

The following case is reported because it presents some peculiar features of a rare condition.

REPORT OF CASE

Clinical History: A male negro, aged 40 years, was admitted to the Los Angeles County Hospital Jan. 15, 1932, with a diagnosis of influenza. He complained of headache, pain in the shoulders and neck, chills, fever and cough. His temperature was 104, pulse rate 100.

In childhood the patient had had measles, mumps, chickenpox and pertussis. He remembered no other illness until Nov. 21, 1931, when he was ill with what he thought was the "flu." He recovered and went to work for a while but in January had similar complaints. He denied venereal disease. By occupation he was a carpenter. He had never used alcohol or tobacco. His father, mother, brother and three sisters were living and well.

Examination revealed a well nourished and well developed negro with a blood pressure of 135/90. There were no significant findings except several carious teeth, tenderness and pain on pressure along the course of the left spinal accessory nerve, and a temperature that fluctuated between 99 and 102 F. The pulse was from 88 to 100.

Blood and spinal fluid Wassermanns were negative. The white blood count was 11,500, polymorphonuclear leukocytes 82 per cent. Urine negative.

The patient left the hospital Feb. 11, 1932, with a diagnosis of left spinal accessory neuritis, folliculitis and pharyngitis.

On May 17, 1932, the patient returned to the hospital complaining of pain in the shoulders, neck, and arms. He said that he had been feeling somewhat better until Feb. 16, 1932, when in trying to stop a gun fight, he fell unconscious and remained so for 16 hours. When he recovered consciousness he found his right arm and right leg were paralyzed. In 3 days he was able to walk with a cane, but the feeling of his right extremities did not return.

Physical examination at this time revealed paralysis of the right leg and arm with marked atrophy from use. There was marked tenderness on attempting to palpate the right iliacus. The legs and ankles were edematous. The blood pressure was 135/90, temperature 99 to 101, pulse 88 to 100.

On June 10, a complaint of extreme tenderness in the right arm over the brachial plexus and in the right latissimus of a pyelitis. On July 24th there was ten-

derness on palpation in both lumbar regions. The next day the patient complained of sudden, severe abdominal pain for which no cause could be detected. On Oct. 3rd he was suddenly stricken with agonizing precordial pain which radiated down the left arm. Morphine was administered and the pain was relieved in about half an hour. The next day there was still residual precordial pain, but not nearly so severe as on the previous day.

Laboratory Findings: Wassermann negative; urine 30 pus cells per field, few casts; basal metabolic rate +6; blood smears negative.

The blood counts were as follows:

May 18, 1932, red blood cells 4,250,000, hemoglobin 75 per cent, white blood cells 7000, polymorphonuclears 60 per cent, eosinophiles 27 per cent, mononuclears 5 per cent, basophiles 3 per cent.

July 26, 1932, red blood cells 3,220,000, hemoglobin 47 per cent, white blood cells 12,500, polymorphonuclears 64 per cent, eosinophiles 15 per cent, lymphocytes 16 per cent, mononuclears 5 per cent.

Aug. 23, 1932, white blood cells 16,700, polymorphonuclears 38 per cent, eosinophiles 33 per cent, lymphocytes 27 per cent, mononuclears 2 per cent.

X-ray examination on Aug. 22, 1932 showed no parenchymal pathology in either lung. Moderate enlargement of the left ventricle consistent with hypertensive heart disease was present.

On May 24, 1932, kidney, ureters and bladder studies were not significant of any renal lesion.

On Oct. 8, 1932, the patient left the hospital against his physician's advice. At least twelve special consultants had examined him but no satisfactory diagnosis could be agreed upon. Suggestions included coronary sclerosis, subdiaphragmatic abscess, echinococcal liver cyst, tuberculosis, coccidioidal granuloma, pyelonephritis, Malta fever. Practically all of these possibilities were ruled out conclusively while the patient was hospitalized.

On Nov. 23, 1932, the patient was readmitted for the third time and was brought to the hospital in a comatose condition. He was emaciated, extremely dyspneic and his heart tones were barely audible. He died 1 hour after admission.

SUMMARY OF AUTOPSY

About the base of the cerebellum and around the brain stem the leptomeninges were greatly thickened. The affected area had a greenish gray color that gave it the appearance of being an acute process superimposed upon a more chronic lesion. The entire area was limited to the base of the brain and was thus quite similar to a tuberculous meningitis, but careful search revealed no miliary tubercles.

A few pleural adhesions were found at the left apex but no tuberculosis was in evidence. There was marked edema of the lungs.

A recent fibrinous pericarditis involved the entire pericardial sac and considerable serosanguinous fluid was present. The heart weighed 460 gm., the increase in size being due to left ventricular

hypertrophy. The myocardium was light brown in color and no areas of fibrosis were found. The aortic cusp of the mitral valve had a soft vegetation 5 mm. in diameter attached to the line of contact. The coronary arteries were quite markedly sclerotic but not occluded.

A small infarct was found in the spleen.

The kidneys presented a striking appearance. Each weighed 290 gm., and on section showed a diffusely granular appearance. The capsule was adherent and when stripped left a granular surface. The cortex was thicker than usual.

The adrenals, pancreas, gastro-intestinal tract, bladder and prostate presented no evidence of gross pathology.

A smear taken from the meninges showed numerous pneumococci.

MICROSCOPIC EXAMINATION

Meninges: There is a marked acute purulent meningitis which overshadows any other pathological condition that might be present.

Heart: The pericardial surface is greatly thickened and infiltrated with large numbers of polymorphonuclear leukocytes, plasma cells and eosinophiles. A small amount of fibrin is present. The small branches of the coronary arteries show a striking change. There is considerable periarterial cellular infiltration of leukocytes, including eosinophiles and plasma cells, which in many instances takes a peculiar bipolar arrangement seen in the photomicrographs. Even more striking is the marked medial and intimal thickening with almost total occlusion of the vessel lumen. The myocardial fibers show surprisingly little evidence of degeneration.

Kidneys: The process in the kidneys is similar to that in the heart but much more pronounced, so that the entire interstitial structure is infiltrated with numerous polymorphonuclear leukocytes, eosinophiles, and fewer plasma cells. The glomeruli and tubules show little change. The arterioles of all sizes are involved and there is more periarterial infiltration than was seen in the heart. Another feature not seen in previous sections is the presence of numerous giant cells around the vessels. These are small in size and contain six to eight relatively large nuclei. The destruction and separation of the various layers of the media are especially well demonstrated in this section.

Liver: Only a few of the larger arterioles show characteristic changes.

Spleen: A typical anemic infarct is found. There is no evidence of polyarteritis.

Pancreas: In the section studied there is one small artery showing changes similar to those noted in the heart and kidney.

Pathological Diagnosis: From the microscopic examination, which, unfortunately, is somewhat incomplete (due to the diagnosis not being suspected at autopsy so that only routine tissue blocks were saved for microscopic examination), this is a case of polyarteritis nodosa affecting chiefly the kidneys and the heart.

DISCUSSION

In this case our failure to make a correct diagnosis of the condition present is instructive enough to deserve some emphasis. Here was a man dramatically ill over a period of nearly 1 year, during most of this period surrounded with competent medical talent, and with all desirable facilities. Yet, as far as we know, polyarteritis nodosa was not once mentioned as a possible explanation of his illness. Even if it had been considered it is doubtful if the diagnosis would have been verified ante mortem, or if the course of the disease could have been altered by its recognition. But this does not justify our failure to consider the possibility of polyarteritis nodosa. It is true that the disease is rare and the clinical symptoms vary, so that we cannot expect positive ante mortem diagnoses in a large percentage of cases. However, as we learn more of its behavior we should come to include it more frequently in our differential consideration of obscure sepses.

Even at autopsy the condition was not suspected. This was due to the fact that in this case only the very small arteries were involved, so that the characteristic nodulations were not noticeable in the gross. Only on microscopic examination of tissue blocks was the positive diagnosis revealed.

Arkin lists the important symptoms observed in his 5 cases as accelerated regular pulse in 5 instances, edema of the legs in 5, septic type of temperature in 4, pain in the extremities, polyneuritis in 4, hematuria in 4, cardiac insufficiency in 3, melena in 3, cerebral symptoms in 2, onset with acute angina in 2, abdominal pain in 2,

and changes in the fundus oculi in 1 case. The clinical findings in our case correspond quite closely with this list, *viz.*, accelerated regular pulse, edema of the legs, septic temperature, pain in the extremities, an acute anginoid syndrome, abdominal pain, a hemiplegic attack, costovertebral pain and tenderness, secondary type of anemia, leukocytosis with eosinophilia, and progressive emaciation.

In all probability the hemiplegic attack that occurred in February, 1932, was due to cerebral arteriopathy, which our histological investigations were not thorough enough to discover. Meningitis has been observed in polyarteritis nodosa, but in this case the meningitis was probably a terminal acute pneumococcic invasion entirely unrelated to the arteritis.

The most striking laboratory finding was the eosinophilia noted in repeated blood examinations. Eosinophilia, however, has not been stressed by other writers and probably is by no means a criterion of the disease.

Polyarteritis nodosa is generally regarded as a progressive and incurable disease. This view is supported by the rapidly fatal termination of most of the reported cases, nearly all of which reveal histological evidences of acute inflammation as well as chronic reparative changes. In spite of this dubious prognosis, there can be no doubt that occasionally the process comes to a halt. Arkin¹⁴ described 1 case of complete histological healing. This patient suffered only one acute illness and then lived 4 symptom-free years before death.

At present it is impossible to judge if any form of therapy can retard the inflammatory changes or assist the healing processes. However, Carling and Hicks¹⁸ used arsenical preparations intravenously and observed consequent remission of symptoms, and this was strikingly confirmed in a recent report by Schottstaedt.¹⁹

SUMMARY AND CONCLUSIONS

The term "periarteritis nodosa" does not accurately connote the morphological realities of the disease as we now know them. Dickson suggested "polyarteritis nodosa" as a name for this condition, which seems a more descriptive term, free of misleading implications.

A specific filterable virus with a selective affinity for the small

and medium sized muscular type arteries of the body is probably the cause of polyarteritis nodosa. Any organ or combination of organs may be affected at any time in the course of the disease, and the resulting clinical manifestations may be bizarre in the extreme. The visceral arteries are involved more frequently than those of the extremities, and the organs most commonly affected are the kidneys, heart, gastro-intestinal tract, pancreas, muscles, peripheral nerves, liver, spleen, and cerebrum.

Pathologically the inflammatory changes are not confined to the adventitia and periarterial connective tissue, as originally supposed. All the vascular coats are eventually involved and the primary changes take place in the media. Destruction of the media may give rise to aneurysm formation. Involvement of the intima with rupture of the elastic membrane may produce thrombosis. The process as a rule is progressive and in practically all of the reported cases there has been evidence of acute inflammatory changes superimposed upon the chronic reparative efforts. However, Arkin has described 1 case of histological healing and he believes that in rare instances the process may come to a complete standstill.

Polyarteritis nodosa is seldom diagnosed or even suspected before autopsy, and even at autopsy there may be no gross indications of its presence. The internist should be familiar with the cardinal symptoms of the disease and its notoriously capricious behavior. Then, when the commoner possibilities have been carefully ruled out in a patient with septic manifestations and varied symptomatology, polyarteritis nodosa should be given consideration.

Carling and Hicks, and recently Schottstaedt have reported cases in which remission of symptoms seemed to follow the intravenous administration of arsenicals.

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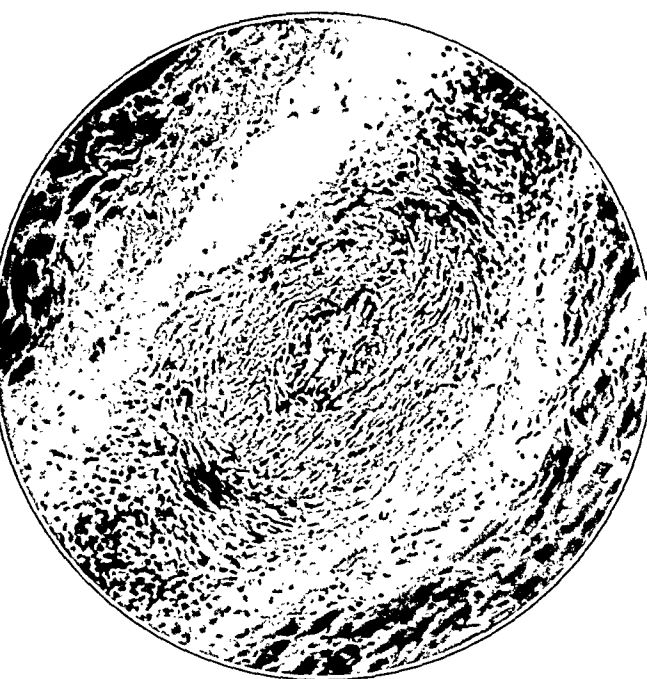
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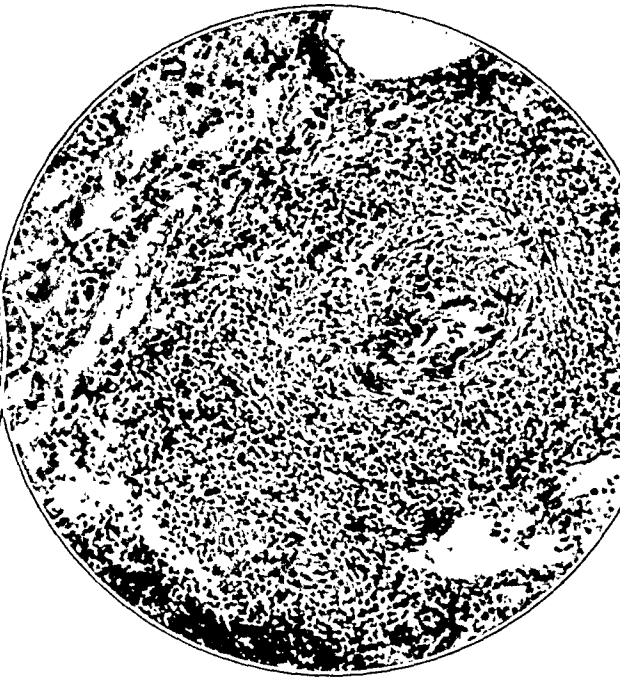
DESCRIPTION OF PLATES

PLATE 95

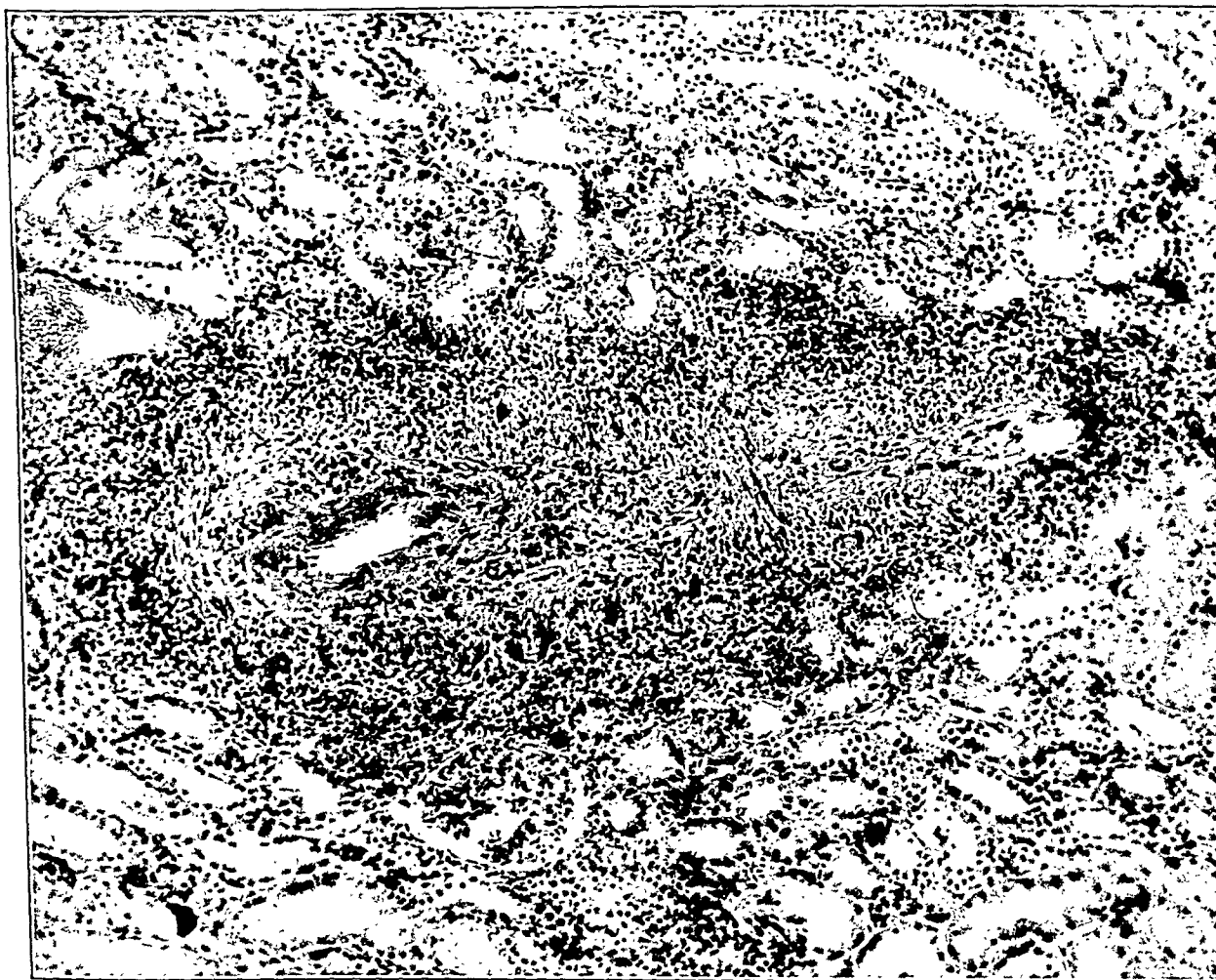
- FIG. 1. Section from heart showing peculiar bipolar distribution of periarterial exudate. $\times 130$.
- FIG. 2. Arteriole of kidney showing intense cellular infiltration with separation of muscle layers. $\times 130$.
- FIG. 3. Arteriole of kidney showing marked thickening of the wall and giant cell formation. $\times 130$.



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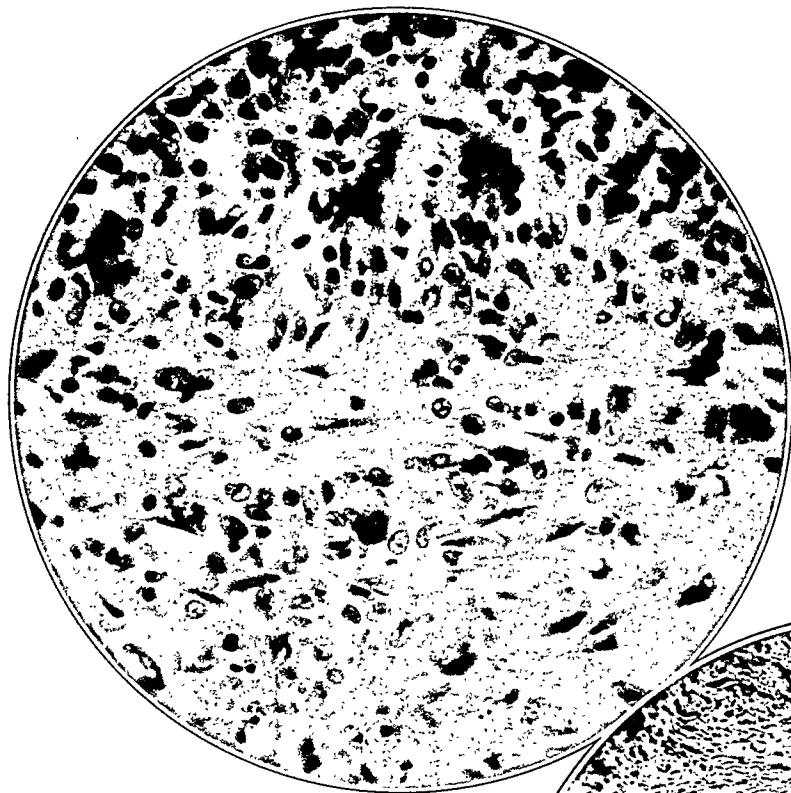
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PLATE 96

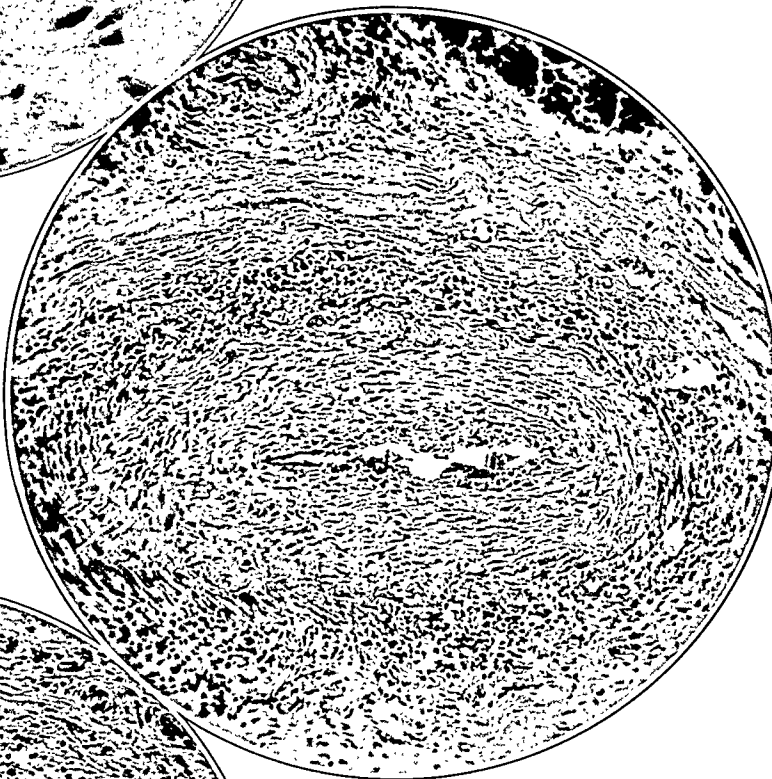
FIG. 4. High power view of giant cells seen in Fig. 3. $\times 250$.

FIG. 5. Small artery in pancreas showing extreme intimal thickening. $\times 130$.

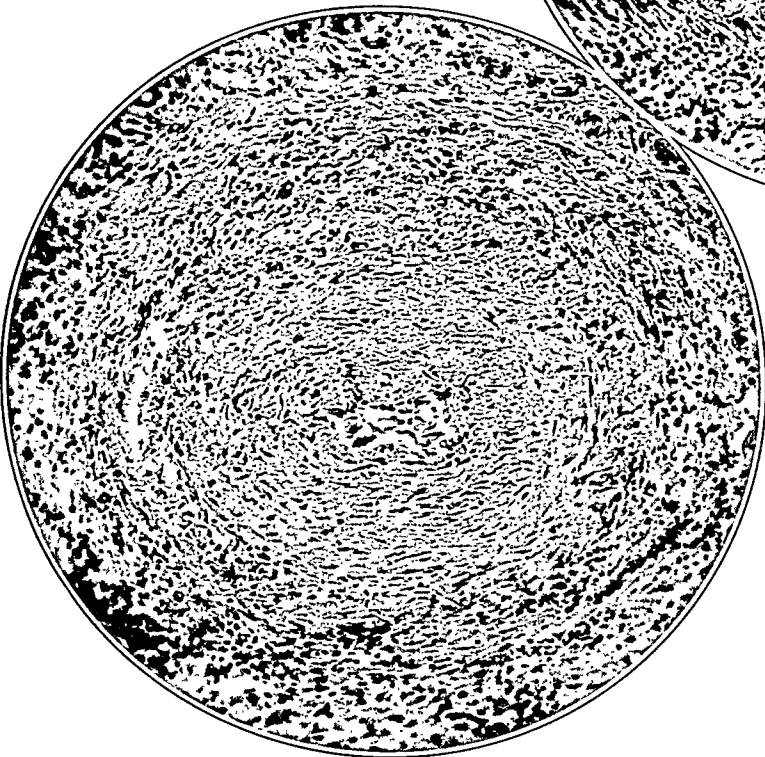
FIG. 6. Arteriole of liver showing extreme intimal thickening. $\times 130$.



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6

HISTOLOGICAL CHANGES IN THE CENTRAL NERVOUS SYSTEM FOLLOWING EQUINE ENCEPHALOMYELITIS *

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REVIEW OF LITERATURE

Meyer, Haring and Howitt ¹ have described an infectious disease in horses and mules which they demonstrated to be produced by a filterable virus, and which affects the brain and spinal cord of these animals. They reported no important gross anatomical lesions, but described significant microscopic changes in the brain and cord consisting of perivascular hemorrhages, infiltration of the sheaths of the blood vessels and scattered patches of leukocytic infiltration in the gray, and occasionally in the white, matter.

Haring, Howarth and Meyer ² have described briefly the symptoms of this disease in horses and mules, which can be attributed directly to the microscopic changes in the brain. They stated that the malady is apparently identical with the horse disease that at different times during the past 60 years has caused heavy losses in various parts of the United States, particularly in the west central states, and which has been called Kansas-Nebraska horse plague, and also, incorrectly, cerebrospinal meningitis, forage-poisoning and botulism.

In a subsequent paper Meyer, Haring and Howitt ³ concluded that the disease, as observed in California, differed etiologically from the equine encephalitis described by Moussu and Marchand ⁴ in France, and from the well known Borna disease of Germany. According to Records, ⁵ and Records and Vawter ⁶ the outbreak, which started in horses and mules in California in 1930, had spread by 1932 throughout most of the western states. Newsome ⁷ described its occurrence in Colorado. In 1932 and again in 1933 Meyer ^{8, 9} summarized the existing knowledge of the disease.

Howitt ¹⁰ has shown that the virus may be recovered during the febrile period from various parts of the central nervous system and

* Received for publication October 23, 1933.

from the blood of experimentally infected animals, including the horse.

Records and Vawter¹¹ have attributed favorable results to the use of antiserums in both laboratory and field cases.

Vawter and Records,¹² Kelser,¹³ and Giltner and Shahan¹⁴ have described various ways of artificially transmitting the disease.

Recently Syverton, Cox and Olitsky¹⁵ have shown similarities in the virus of equine encephalomyelitis and vesicular stomatitis, when inoculated into monkeys, guinea pigs and mice. They found that when such animals succumbed to experimental encephalomyelitis or vesicular stomatitis, the gross and microscopic changes in the brain were apparently identical and the same type of intranuclear inclusion body was formed in the neurones. They state that similar tissue changes occurred in the liver and kidney.

The particle size of encephalomyelitis virus in brain suspensions has been estimated at 500μ by Krueger, Howitt and Zeilov.¹⁶ They state that under like conditions of preparation and filtration it is of the same order of magnitude as the causal agent of poliomyelitis and apparently ten times the size of the hoof and mouth disease particle.

MATERIAL AND METHODS

Intensive microscopic study of the brains and spinal cords of eight horses affected by the virus of encephalomyelitis, either experimentally or by contagion, has yielded additional information as to the effects on the nervous system. As controls we have studied the brains and cords of three horses that died from sepsis with no clinical signs of encephalomyelitis, and the brain and cord of one normal horse that was killed by exsanguination. In addition the brains of two guinea pigs injected with filtered virus and killed with chloroform 3 and 4 days after successful inoculation were studied by the same methods. Also sections from various parts of the brain of a farmhand who died with symptoms similar to those of the equine encephalomyelitis, to which he had been exposed, were studied.

Portions of the brains and spinal cords of the experimental animals were fixed immediately after death in 10 per cent formalin, in formol-Zenker solution, or in Bouin's fluid. Material from the field animals was fixed in some cases shortly after and in other cases many hours after death, and was less satisfactory for histological study. The human brain was obtained at autopsy 12 hours after death and fixed in formalin.

Pieces from various parts of the nervous system were embedded in paraffin and sectioned at 5 microns. Sections were stained with Loeffler's methylene blue and basic fuchsin, or by Maximow's hematoxylin and eosin azur method. Prior to staining many of the sections were treated on the slide by immersion for 1 to 2 hours in Zenker's fluid and then washed with tincture of iodine for

removal of any precipitate of mercuric chloride. This treatment increased the stainability of the sections, especially of material fixed in formalin.

Other sections from a number of animals were subjected to tests for various pigments, since it was apparent early in the study that diffuse blood pigments in the tissues, and other pigments, especially in the large nerve cells of the brain stems of many of the horses, led to much confusion. It therefore became necessary to take into consideration the age of the animals and to test some of the material for blood pigments, lipochromes and melanin pigments. The tests applied were the alkaline alcohol treatment for blood pigments, the ether and chloroform solubility test for lipochromes and lipofuscin, the osmic acid color tests for lipochromes, and the osmic acid and silver nitrate tests for melanins. The sections subjected to these various procedures were afterward stained lightly with hematoxylin and eosin or with safranin. Through the courteous coöperation of Dr. Gordon H. Scott, of the department of cytology of the Washington University Medical School, the method of incineration on the slide was also used.

The tests for lipochromes leave much to be desired, since they could be applied only on material already subjected to the action of various fat solvents used in preparing paraffin sections. The combined results of the several tests, however, are worthy of some consideration and are described later. Lipofuscins are usually regarded as resistant to solution with alcohol after fixation other than directly with fat solvents. This fact, together with the yellow to yellowish green color of many of the inclusion granules, is considered indicative of lipofuscins.

MICROSCOPIC EXAMINATION

Sections of the brain and spinal cord of horses affected by the virus show the perivascular and leukocytic infiltration, in greater or less degree, already mentioned (Fig. 1). The cell types in the perivascular cuffs are lymphocytes, polymorphonuclear leukocytes and monocytes. In some of the horses many cells are phagocytic, containing numerous granules of brown pigment. Granule-laden phagocytes are also present within the blood vessels in a number of animals. The perivascular cuffs continue along the blood vessels for considerable distances and in some cases can be followed to the brain surface. Outside the layer of infiltrated cells there is a clear zone of varying width which in part, at least, represents shrinkage of the edematous brains, due to treatment with alcohol. The sections from most of the animals indicate considerable edema, especially in the brain stem and cortex. Diffuse blood pigments are present along the course of the blood vessels. These disappear on treatment with alkaline alcohol, as do the pigment granules in the phagocytic cells. The latter are accordingly also interpreted as blood pigments.

The control horses, three of which died from sepsis and one of which was killed, show no perivascular infiltration of cells. One of

the three dying from sepsis was not exsanguinated and shows an unusual amount of blood pigment in brain and cord. The two guinea pigs show no blood pigment, but both have perivascular infiltration, the one killed 4 days after inoculation in more marked degree than the other killed 3 days after being inoculated with virus. In the one human brain examined perivascular infiltration is also present, but little blood pigment is apparent. This brain was very edematous, as judged from the sections.

Many of the large nerve cells of the brain stem and spinal cord, and to a less extent in various parts of the cerebral cortex and cerebellum, show cytoplasmic inclusions in much larger numbers than in nerve cells of animals dying from other causes, as judged from the four control horses examined by us and from the large number of horses ranging in age from 2½ months to 30 years, reported by Kikuchi.¹⁷ The inclusions consist of granule-like bodies which vary from 0.3 to 0.7 microns in diameter. Most of them are rounded but many of the larger ones are somewhat angular in form. In staining properties they show a wide range of variation which appears to depend in part upon the method of fixation and the state of the tissue when placed in fixing fluid. In material fixed in formol-Zenker's fluid immediately after death of the animal the eosin of the Maximow stain gives them a deep pink tint. In sections from blocks fixed in formalin or in Bouin's fluid they fail to stain with Maximow's technique unless previously treated on the slide with Zenker's fluid, and then they show much variability in staining reaction. Usually, with this treatment, many of the granules take a pink tint, but many others remain nearly colorless or yellowish. In the same cell frequently are found granules ranging from pale yellow to pink in color. In much of the material yellowish green to green granules are present, especially after treatment with Zenker's fluid followed by iodine. This suggested that the granules are related to lipochromes and led to further tests for lipochromes and other pigments, as described in another part of this report in further detail.

The basic fuchsin stain colors the granules a brick red, irrespective of fixation. This stain was useful in locating groups of affected cells but was of little value otherwise, as compared with the other methods employed.

The distribution of affected cells in sections of material most

favorable for careful microscopic study is somewhat irregular. There are considerable areas of apparently normal cells, while at the margins of such areas are found groups of nerve cells showing inclusions and various stages of cell degeneration. In other sections considerable areas of affected cells occur, varying from nearly normal ones to cells in the last stages of disintegration. In general, nerve cells near blood vessels have progressed farthest in the degenerative process, suggesting that the destructive agent is brought by the vessels or along their sheaths.

PHAGOCYTOSIS OF NERVE CELLS

Some of the injured nerve cells show the effects of marked activity of phagocytes (Figs. 3 and 7). Such cells are necrotic with shrunken eccentric nuclei whose nucleoplasm is basophilic and whose nuclear membrane usually has disappeared. The cytoplasm of these cells is shrunken and altered and Nissl bodies are markedly chromatolytic or absent. Inclusion bodies, however, are usually demonstrable. They have the same staining reaction and position as in slightly damaged cells. Phagocytes frequently may be seen burrowing into the surface of the nerve cells from all sides (Fig. 3) and affecting both perikaryon and cell processes. Extreme cases are encountered where only fragments of the nerve cells remain, surrounded by phagocytes. No phagocytes have been found deeply embedded within the nerve cells, as described by Da Fano and Ingleby¹⁸ (see page 358), but the burrowing action of phagocytic cells is evident from Figure 3.

Many nerve cells, in which nuclei are still nearly or quite normal in appearance, show small vacuoles in the outer layers of their cytoplasm (Fig. 3). The vacuoles vary somewhat in size and distribution. Inclusion granules present in such cells lie in the deeper part of the cytoplasm. There is no apparent direct relation between vacuoles and granules.

NUCLEI OF AFFECTED NERVE CELLS

The fuchsinophile nuclear inclusions found by Joest¹⁹ in the nerve cells of Borna's disease of horses, a disease that has many points of similarity to the horse encephalomyelitis of Haring and Meyer, naturally led to a careful search for intranuclear bodies in our material. Some cell nuclei in horse material (A147 and A252,

fixed immediately after death) contain rounded bodies of about the same size as those in the cytoplasm but, as a rule, of bluish color. Occasionally acidophilic granules appear in the nuclei. In animal A 41, a pregnant female 6 years old, the Maximow stain reveals reddish granules in the nuclei of many of the nerve cells. The granules are indistinct individually, but give a reddish tint to the nucleus. In this animal the cytoplasmic inclusions are small and are largely hidden by the Nissl substance. There was also but little perivascular infiltration in this animal, although clinically it had encephalomyelitis.

In guinea pigs injected with filtered virus numerous reddish granules are brought out by the Maximow method in many nerve cell nuclei, but most of the nuclei are clear and normal in appearance, even when the cells contain cytoplasmic inclusions. In these guinea pigs the cytoplasmic granules, as in horse A 41, are small and more difficult to find than in most of the horse material. The greater part of the material examined, however, including the human, shows very little indication of anything but the normal chromatin granules in the nuclei by the methods employed. In view of the reported discovery by Syverton, Cox and Olitsky¹⁵ of intranuclear inclusions in various animals subjected to the virus of equine encephalomyelitis, our failure to find nuclear inclusions constantly may be due to variations in the condition of the tissue when placed in fixing fluid, caused by differences of the time interval after death, and to other factors of technique which were not controllable in field animals and in human autopsy material. Many nuclei are found in various stages of destruction, with loss of nuclear membrane, and with acidophilic nucleoplasm, but only in markedly chromatolytic and otherwise necrotic appearing cells.

CYTOPLASMIC INCLUSIONS

The inclusion bodies in the nerve cells occur, as a rule, as clusters of granules near the nucleus of the cell. Most frequently they are found at one side of the nucleus (Figs. 2, 3 and 5). Sometimes they occur in several clusters, which may be situated at opposite poles of the nucleus (Figs. 4 and 6). Not infrequently the granules are scattered throughout the cytoplasm without reference to the nucleus. In the larger nerve cells they have been encountered in clusters in

the dendritic processes at considerable distances from the perikaryon. Occasionally they occur as compact, well circumscribed masses of granules, either near the nucleus or elsewhere in the cytoplasm.

Such inclusions are found in nerve cells that show only slight indications of degeneration (Fig. 4), and they also are present in cells that are in the last stages of destruction and that are undergoing phagocytosis (Fig. 3). The hematoxylin-eosin azur method is particularly favorable for staining these granules and the Nissl substance at the same time, when the latter still remains. The Nissl granules take a grayish blue tint, in marked contrast to the unstained, or pink inclusions. In Figure 4 the large, flaky Nissl bodies are shown throughout the perikaryon, appearing in the photomicrograph as gray masses. The inclusion granules, somewhat masked by the Nissl bodies, are shown in the photomicrograph as smaller black dots at either pole of the nucleus. The nucleus itself has been but slightly affected in the cell shown in this figure. Another cell is shown in Figure 5, in which Nissl bodies still are present at the periphery of the perikaryon, but in which chromatolysis has progressed much farther. The nucleus also is pyknotic and eccentric in position. The inclusion granules, however, are altogether similar to those of Figure 2, and show more clearly in the photomicrograph because they are not partially hidden by Nissl bodies. The nerve cells shown in Figure 6 have degenerated markedly, but the inclusion bodies have the same appearance as in the other cells.

Many publications have appeared on intracellular and intranuclear inclusions in relation especially to virus disease. Findlay and Ludford²⁰ have reviewed this literature up to 1926 and Cowdry²¹ has again covered the field up to 1927. Another survey is, therefore, unnecessary.

Hueck²² has differentiated lipofuscin from other lipid inclusions in nerve cells and other tissues. He states that there is considerable variation in the amount of lipofuscin in different types of nerve cells. Kikuchi,¹⁷ as already noted, in 1928 described lipofuscin and other inclusions in the nerve cells of horses ranging widely in age. He considers lipofuscin to be a normal constituent of the nerve cells, but shows that it is more abundant in older animals. Bielschowsky,²³ agreeing in general that lipofuscin is present in normal nerve cells, holds that under certain pathological conditions there is a great in-

crease of this pigment. The nucleus in cells undergoing such pathological formation of pigments may remain well preserved for a long time but eventually succumbs, preliminary to destruction of the cell.

Da Fano^{24, 25} has described minute bodies in herpetic encephalitis of rabbits. So far as can be judged from his descriptions and excellent figures these minute bodies are probably not identical with the cellular inclusions of the present account. Da Fano found bodies in various cells of the brain, including nerve cells. In our material the granules are found only in the nerve cells. There are also certain differences in the appearance of the granules themselves, as compared with the minute bodies of Da Fano. The latter are described²⁴ (see page 97) as a "single or double minute granule, roundish or slightly oval, surrounded by a clear halo; when in pairs the granules are so closely attached one to the other as to convey the impression of dumb-bell shapes; these are surrounded by a common and somewhat wider halo." Only occasional granules in our material show any sign of a halo and there are few if any double granules. In form the range in our material is from rounded to angular, as above described. Da Fano's minute bodies, furthermore, stain a deep purple-blue or purple-red with polychrome methylene blue and Giemsa's stains. Subsequently Da Fano²⁵ and Da Fano and Ingleby¹⁸ reported minute bodies in epidemic encephalitis in man, but with negative results as respects their presence in nerve cells.

Cowdry and Nicholson²⁶ describe and figure inclusions in the nerve cells of rabbits subjected to herpetic virus, which in size and general characteristics are not unlike those here described. In staining reaction, however, the granules of these investigators were colored a deep blue by the Giemsa stain on air-dried films.

It is apparent from Table I that there are at least three, and probably four, kinds of pigment in some of the brains studied by us. The blood pigments, while confusing because of their wide diffusion in the sections, are easily eliminated by the alkaline alcohol treatment. The melanin pigments can usually be recognized without difficulty in hematoxylin and eosin sections, and on treatment with silver nitrate they show the blackening that is characteristic of this test. With osmic acid they assume a deeper brown coloration. They are not dissolved in ether and chloroform. In a normal horse, 12 years of age, such granules are present in considerable numbers in the

TABLE I

Results of Various Microchemical Tests Applied to the Cytoplasmic Inclusion Bodies

Material	Age	Brain part	Silver nitrate	Osmic acid	Ether and chloroform	2% Sodium hydroxide in 80% alcohol	Incineration at 600° C
Normal horse	3 ^{rs} . 12	Medulla oblongata Midbrain	Black granules Black granules	Brown granules			
Horse No. A147	Aged	Various parts	Yellow granules	Dark brown granules	Pale to yellowish granules	Blood pigments dissolved, other granules present	Granules disappear save for a slight ash
Horse No. A252	16	Various parts	Yellow granules	Dark brown granules	Yellow granules	Blood pigment dissolved, pale granules in nerve cells	Granules disappear save for a slight ash
Horse No. 1683	Young	Midbrain	Some black granules	Brown granules	Granules faint to fairly distinct		
Horse No. 1684	Young	Midbrain Olfactory bulb	Yellow to black granules Diffuse precipitate	Brown granules	Yellowish granules Pale granules		
Human No. 1513	43	Cerebral cortex Deep cerebellar nuclei Purkinje cells Hippocampus	No blackening Some black granules No blackening	Brown granules, vacuoles Yellow and brown granules Light brown granules Brown granules, some vacuoles	Yellow to brown granules Brown granules Pale granules, some solution Brown pigment	No blood pigment, pale granules in nerve cells No blood pigment No blood pigment No blood pigment, pale granules in nerve cells	

brain stem. Horses A147 and A252, although older than the normal horse, do not show any brown granules except after treatment with osmic acid. In sections treated with silver nitrate only yellowish granules are visible in the nerve cells. Treatment with ether and chloroform is followed by some indication of solution of some of the inclusions, but most of the pale to yellowish granules still remain. The poor solubility and the color of these inclusions point to lipofuscins. On incineration of thin unstained sections, mounted on the slide with distilled water only and subjected to a temperature of 600° C in an electric oven, the organic substance of the cells, including the granules, entirely disappears. Only a reddish ash suggesting iron is left in the position of the granules, according to Dr. Scott, to whose generous coöperation we owe the application and interpretation of this test. The younger horses, Nos. 1683 and 1684, show some melanin in the midbrain but very little if any elsewhere. Inclusion bodies are widely distributed, however, in this material, appearing as yellow granules after all the tests, save the osmic acid which turns them brown.

Sections from various parts of the human brain which had been fixed in formalin show a considerable range of reactions to the tests applied. Only in the deep cerebellar nuclei are there granules that give positive indications of melanin, but sections through the deep part of the midbrain were not available. Cerebral cortex, hippocampus and the Purkinje cells show yellow to brown granules, with some solution by ether and chloroform. The yellowish green to green color of granules observed in some of the horse material was also observed in some of the human nerve cells.

The results of the tests as to the nature of the inclusions point in the direction of lipochromes and lipofuscin. The variations in staining qualities appear to depend not only upon the stain employed and the preliminary treatment, but also upon changes that must take place within the granules themselves, as shown by the presence in many cells of granules that range from pale yellow to deep pink in color when treated by Maximow's eosin azur stain. There is, however, no apparent relation between the stage of destruction of the nerve cells containing them and the staining qualities of the granules, since in material fixed immediately after the death of the animals, *e.g.*, horses A147 and A252, nerve cells in various stages of necrosis are present in which no differences are apparent in the granules.

SUMMARY AND CONCLUSIONS

Histological and cytological study of the brain and spinal cord of horses, guinea pigs and humans subjected to the virus of equine encephalomyelitis shows characteristic pathological changes. The most constant feature is the perivascular infiltration already pointed out by Meyer and Haring. This is found in all horses affected by the virus, but in none of the controls. It is also present in the guinea pig and human brains that were affected by the virus.

There are suggestions of intranuclear inclusions in some of the nerve cells in several animals, but in our material this feature is too inconstant to permit of considering them as characteristic features of the affected cells.

There is considerable degeneration of Nissl substance in many nerve cells of virus-infected animals and also in the human brain. Nerve cells in various stages of necrosis are present, especially in the brain stem and spinal cord. Many nerve cells are in process of phagocytosis by leukocytes.

Cytoplasmic inclusions are present in many nerve cells of all the animals studied, which were affected by the virus, and in the human brain. Similar inclusions are found in smaller numbers in three horses that died from an unknown sepsis and also in a normal horse 12 years of age. The number of inclusions in the nerve cells of the virus-infected animals is considerably greater than in the control animals, and appears to be increased by the pathological conditions of the disease.

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DESCRIPTION OF PLATES

PLATE 97

FIG. 1A. Photomicrograph showing the perivascular infiltration following encephalomyelitis in the brain of a horse.

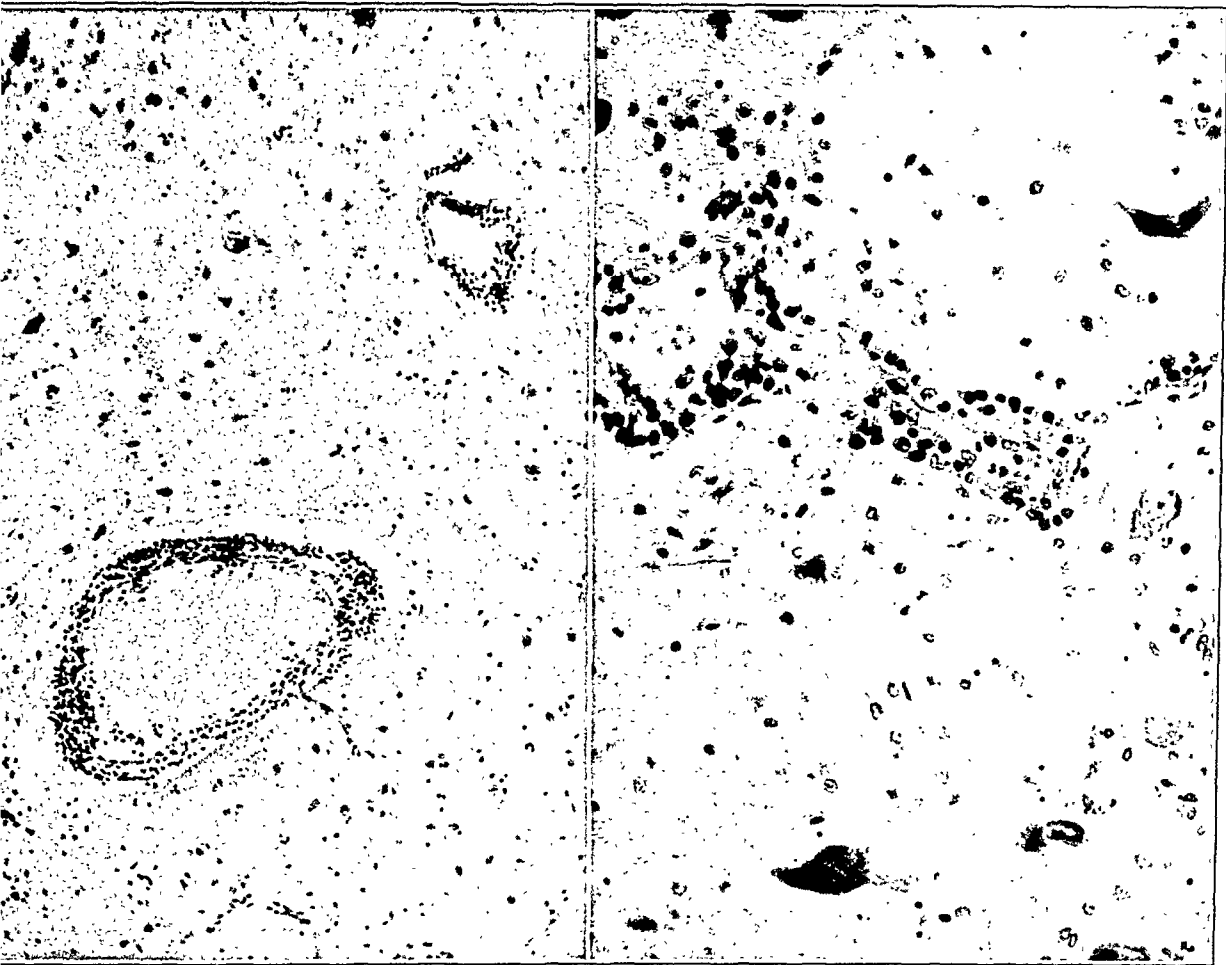
FIG. 1B. Photomicrograph with higher power showing perivascular infiltration and also nerve cells in various stages of necrosis.

FIG. 2. Brain stem of horse No. A147, showing nerve cell and small blood vessel with cytoplasmic inclusions in a necrotic cell and perivascular infiltration, respectively. Hematoxylin and eosin azur stain after Zenker fixation. Section 5 microns.

en = endothelium; In = cytoplasmic inclusions; N = Nissl bodies.
× 820.

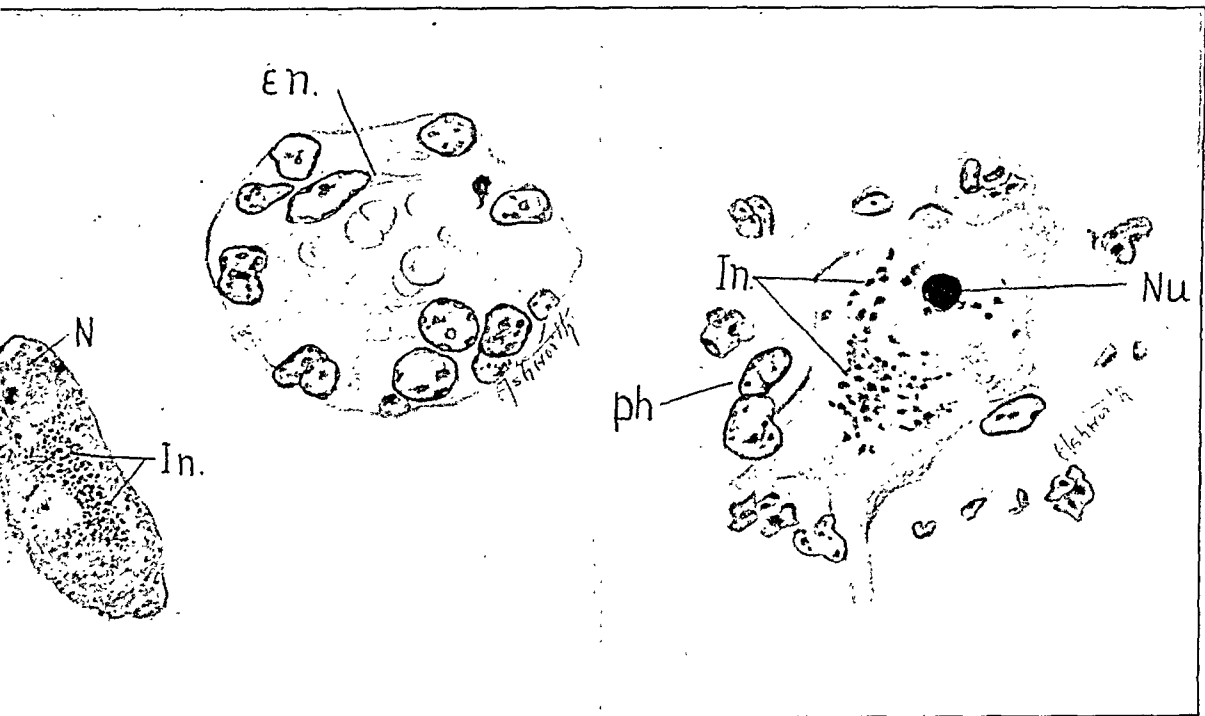
FIG. 3. Horse No. A147. Nerve cell surrounded by phagocytes. The cell is necrotic and also shows inclusions. Hematoxylin and eosin azur stain after Zenker fixation. Section 5 microns.

In = cytoplasmic inclusions; Nu = nucleolus; ph = phagocytes.
× 820.



1A

1B



2

3

PLATE 98

FIG. 4. Horse No. A147. Photomicrograph of nerve cell in brain stem showing Nissl bodies (grayish) and cytoplasmic inclusions (black). Hematoxylin and eosin azur stain after Zenker fixation. Section 5 microns.

In = cytoplasmic inclusions; M = precipitate of mercuric chloride; N = Nissl bodies; Nu = nucleolus.

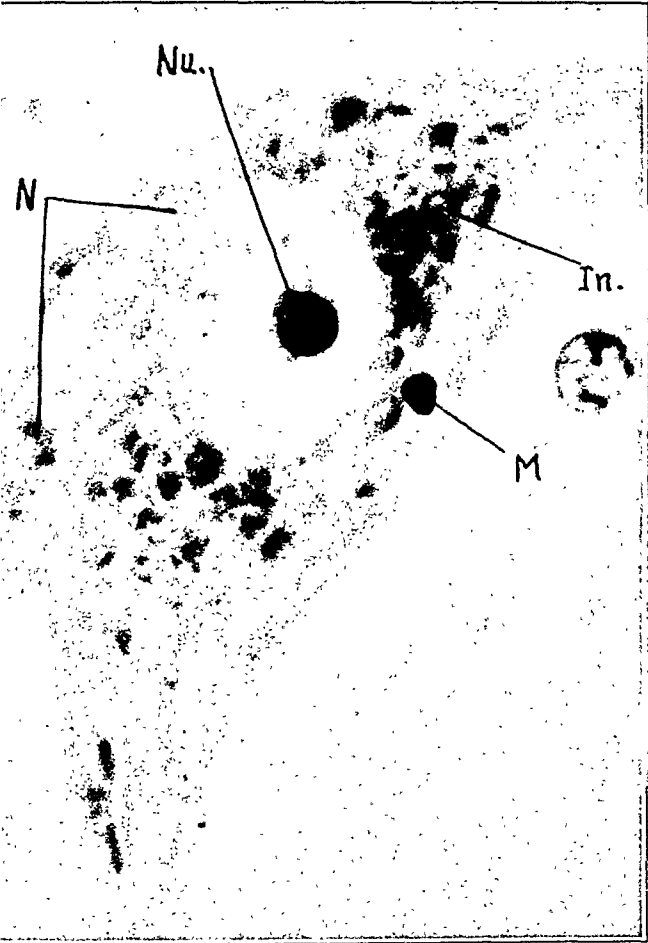
FIG. 5. Horse No. A147. Photomicrograph of nerve cell in later stage of destruction than that shown in Fig. 4. Nissl bodies are found only at the periphery of the cell and the nucleus is eccentric and shows considerable degeneration. Hematoxylin and eosin azur stain after Zenker fixation.

In = cytoplasmic inclusions; M = precipitate of mercuric chloride; N = Nissl bodies; Nu = nucleolus.

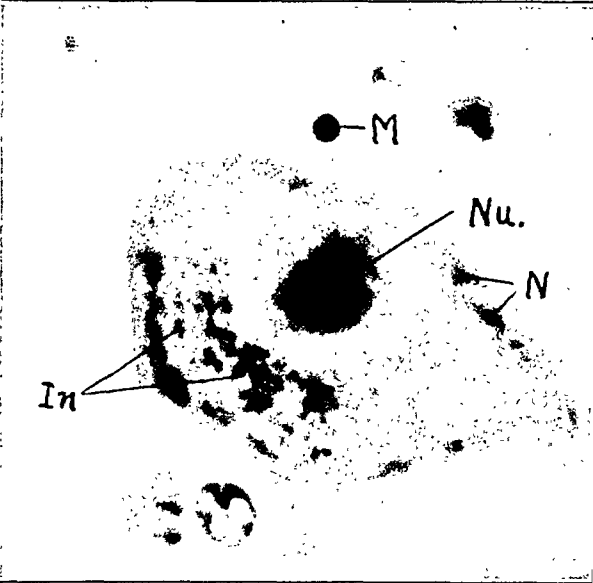
FIG. 6. Photomicrograph of nerve cells markedly degenerated with advanced chromatolysis. Hematoxylin-eosin azur stain after Zenker fixation. Section 5 microns.

In = cytoplasmic inclusions; M = precipitate of mercuric chloride; Nu = nucleolus.

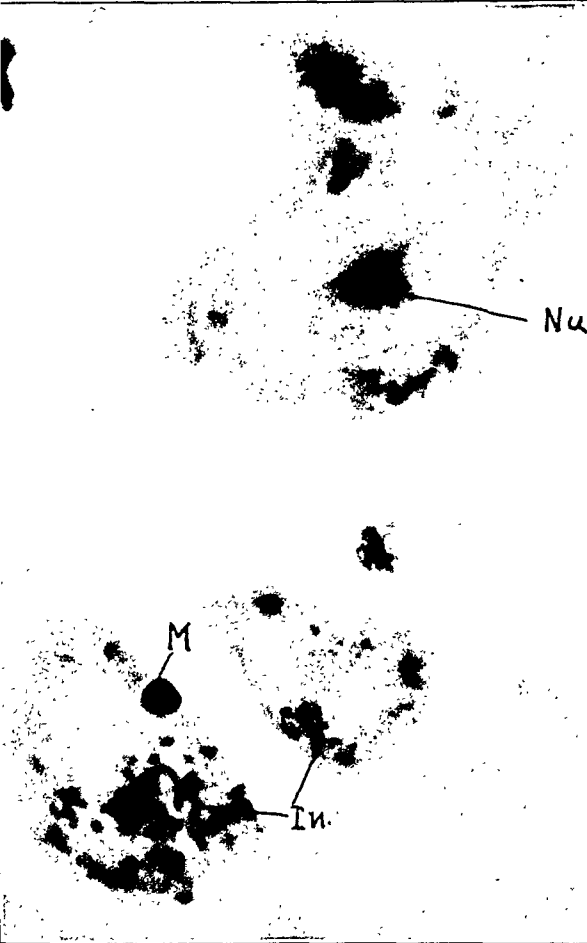
FIG. 7. Brain stem of horse No. A252 showing a group of nerve cells undergoing phagocytosis. Hematoxylin and eosin azur stain after Bouin fixation. Section 10 microns.



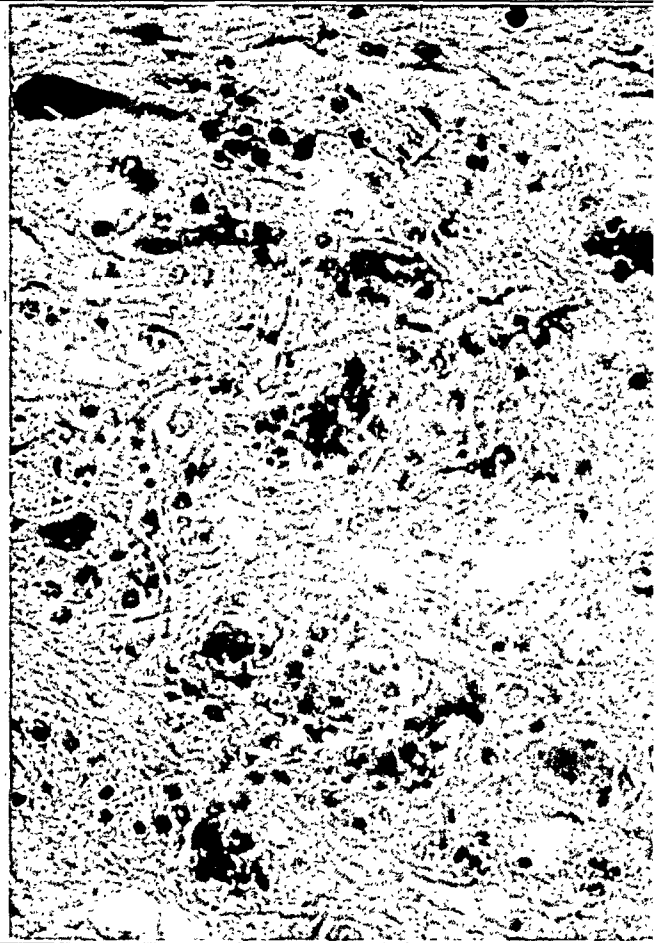
4



5



6



7

ANOMALIES OF THE INTERVENTRICULAR SEPTUM AND PULMONARY ORIFICE *

REPORT OF TWO CASES

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Malformations representing arrests at successive stages in the evolution of the developing heart have recently been observed in this laboratory in 2 cases. Each showed a defect in the interventricular septum which was associated with pulmonary atresia and dextro-position of the aorta in 1, and with stenosis of the pulmonary orifice in the other. The first case is of further interest in that it is one easily mistaken for truncus arteriosus communis persistens, and the second because it suggests the probable pathogenesis of the malformation in this case. Abbott's¹ review of 850 cases of cardiac malformations yielded 186 instances of basal defects of the interventricular septum complicating other anomalies. Among these 95 had pulmonary stenosis or atresia, and associated dextroposition of the aorta was encountered in 62 of the latter group. In view of the rarity of the condition these two cases seemed worthy of reporting.

CASE REPORTS

CASE 1. R. W., an 18 months old negro boy, was admitted to the New Haven Hospital on March 21, 1933, appearing acutely and gravely ill. He had been under observation in the Children's Community Center of New Haven and was known to have congenital heart disease and a spastic right-sided hemiplegia of unexplained origin. According to the mother this was her fifteenth child and all the others were living and well. The mother was 42 years of age. A Kahn test of the blood of both the father and mother was negative. The child, born at term on Oct. 17, 1931, following an uneventful pregnancy, was delivered spontaneously and weighed 4025 gm. The only unusual features noted at birth were "a collodion-like membrane" which peeled off in large pieces from the normal skin, and scant development of the eyebrows and eyelashes. On the 4th day after birth a rise in temperature to over 38° C developed and lasted for about a day and a half. On Oct. 28, 1931, a blue coloration of the lips and nail beds was noted and a double murmur was heard over the entire precordium and the left back. Roentgenographs showed an unusual cardiac silhouette and

* Received for publication October 23, 1933.

a marked enlargement of the left ventricle. The transverse diameter of the cardiac shadow measured about 6 cm., while the intrathoracic diameter at the level of the ninth rib was 10 cm. The mediastinal shadow was normal and there was no prominence of the shadow of the pulmonary artery. When discharged from the hospital the next day the infant weighed 3860 gm.

He was then followed in the Outpatient Clinic and was seen first on Nov. 2, 1931, a few days following his discharge from the hospital. At this time he appeared to be in good health, weighed 4040 gm., and was taking the breast and formula well. When next seen on Nov. 23, 1931, the child weighed 4440 gm. and had a scaly papular lesion on the cheeks, scalp and skin folds. A systolic blow was heard over the left infraclavicular region. A roentgenogram revealed practically no change in the configuration of the cardiac shadow. During the next 2 months the child developed satisfactorily. On Jan. 18, 1932, he weighed 5320 gm. A roentgenogram taken at that time showed a slightly enlarged cardiac silhouette with a transverse diameter of 7.1 cm. and an intrathoracic diameter of 12.5 cm. The pulmonary shadow was not enlarged. On Feb. 8, 1932, the child weighed 5620 gm., showed definite cyanosis of the tips and nails of the fingers and toes, and some dyspnea when in the sitting posture. No murmurs were heard over the heart. In the second interspace, just to the left of the sternum, the first sound was not audible. On March 15, 1932, the weight was 6500 gm. In addition to a moderate degree of cyanosis of the lips, tips of fingers and toes, a widening of the cranial sutures and of the frontal and occipital fontanelles was noted. The circumference of the head was 40.5 cm. When seen on April 19, 1932, the child had a cough and looked rather ill, but his sleep and appetite were not impaired. He weighed 6890 gm. and his temperature was 39° C. A roentgenogram taken on April 22, 1932, showed the lung fields to be clear. There was a marked enlargement of the left ventricle. The transverse diameter of the cardiac shadow measured 7.8 cm. and that of the chest 13.4 cm. He seemed to have recovered from this episode when seen 5 days later. On May 25, 1932, he weighed 7260 gm., and the circumference of his head was 42 cm. On June 8, 1932, the mother noticed a swelling of the right cheek but this seemed to have disappeared when the child was seen again 2 days later. On his next visit, Oct. 14, 1932, the weight was 7120 gm.

He was seen again on Feb. 1, 1933, at which time the mother stated that on the previous day the child became fretful and that he cried and ate and slept poorly. The next day he ate better, was drowsy and awake only for feedings. It was noticed that the child cried when he was picked up by the armpits and that his right arm was limp and "not used." On Feb. 3, 1933, he was admitted to the New Haven Hospital. At this time he appeared thin and small for his age, inactive and drowsy. He weighed 7010 gm. and measured 73.5 cm. in height. The systolic blood pressure was 85 and the diastolic 50 mm. Hg., the pulse rate was 150 per minute, and the temperature was 39° C. There was a slight tachypnea but no obvious respiratory distress. Slight cyanosis of the lips and nail beds, with some clubbing of the fingers and toes, was noted. The circumference of the head was 44.2 cm. The frontal fontanelle was large, open and did not bulge. The frontal bones were prominent. The eyes were kept turned toward the left. The pupils were equal, regular and reacted to light. The ocular fundi appeared normal. The cardiac area seemed not to be enlarged on percussion. A loud systolic murmur was heard over the precordium, was transmitted to the great vessels of the neck, and was heard all over the cranium. A systolic thrill was felt in the suprasternal notch. A roentgenogram disclosed an

enlargement and boot-shaped configuration of the cardiac shadow, as well as an increase in the angulation of the tracheal bifurcation. Both pulmonary fields were clear. The right arm and leg showed a flaccid paralysis. The erythrocyte count was about 6,880,000 with 57 per cent hemoglobin; the white blood cell count was 11,100, with 62 per cent polymorphonuclear leukocytes and 34 per cent lymphocytes on several occasions. The urine contained no albumin or sugar. Roentgenograms of the skull, pelvis and extremities were essentially negative. Roentgenoscopic examination of the esophagus with a barium meal showed no narrowing. A prominence of the shadow of the pulmonary artery was also noted. During the subsequent stay of 5 weeks in the hospital the right hemiparesis gradually improved and the child gained some weight. An electrocardiogram taken on March 3, 1933, showed a sino-auricular tachycardia and left axis deviation (new terminology). When transferred to the Children's Community Center on March 13, 1933, the child weighed 7600 gm. All neurological signs had disappeared, except that the right leg was held flexed at the knee but could be extended voluntarily.

Three days after discharge from the hospital the child again became irritable, took food poorly and in the next few days became progressively weaker. On March 17, 1933, he had a brief episode of spastic quadriplegia. On March 19 he had an elevation of temperature which ranged between 39.4° and 41.2° C. The next day a discoloration was noted along a vein running over the forehead and bridge of the nose. When readmitted to the New Haven Hospital on March 21, 1933, the child appeared acutely and gravely ill and difficult to arouse. He weighed 6900 gm. and his temperature was 37° C. The bluish coloration of the bridge of the nose and forehead was still present. The eyes deviated to the right, showed a fine horizontal nystagmus, and the pupils reacted but little to light. The eyegrounds were essentially normal. There was marked cyanosis of the lips. The gums were reddened. A convulsion was produced when an attempt was made to examine the throat. The lungs were clear. The heart was not enlarged to percussion, its rate was rapid and regular. A blowing systolic murmur was heard loudest over the pulmonic area. A murmur was faintly heard over the right parietal region. Cyanosis and clubbing of the fingers and toes was noted. There was a slight spasticity of all the extremities and the tendon reflexes were more hyperactive on the right than on the left. The erythrocyte count was 8,120,000 with 74 per cent hemoglobin; the white blood cell count was 28,000 with 85 per cent polymorphonuclear leukocytes and 13 per cent lymphocytes. The urine contained no acetone, sugar or albumin. A lumbar puncture on the next day showed clear cerebrospinal fluid. The temperature rose to 39.8° C but was lower the next day and then remained normal with occasional rises to 38° C. Feeding required recourse to gavage, and hypodermoclyses were frequently given. On April 3, 1933, signs of pneumonia set in and on April 5 the child died.

At autopsy, in addition to the anomalies in the heart and large vessels, the following changes were noted: slight clubbing of fingers and toes, large open frontal fontanelle, increased circumference (44 cm.) of the head, hypoplasia of the thymus and spleen, chronic passive congestion of the viscera, bilateral focal pneumonia, heman-giectases of the veins of the right Sylvian fissure, thrombi in the left

cerebral artery and its branches with multiple old and recent infarcts of the brain.

Description of the Heart: *In situ* the heart was not particularly enlarged, its transverse diameter was 8 cm., that of the chest 14 cm. The apex was markedly rounded and made up equally of both ventricles. From the conus arteriosus a single vessel arose which at first sight appeared to be a common trunk for the pulmonary artery and the aorta. The disposition of the inferior vena cava, the azygos vein and superior vena cava, the coronary sinus and its tributaries appeared normal.

The heart weighed about 70 gm. The right half was slightly larger than the left. The right atrium appeared normal. The atrioventricular orifice measured 6.5 cm. in circumference; the valve was not altered. The wall of the right ventricle was 7 mm. thick; the trabeculae carneae and the muscoli papillares were well rounded. There was an opening in the septum ventriculorum in the region of the membranous septum, 1 cm. in diameter, forming a communication between the ventricles. The orifice of the arterial trunk measured 4 cm. in circumference; the valves appeared normal. The left coronary artery arose from the left sinus of Valsalva, the right from the anterior sinus. The subsequent course of each of these vessels appeared normal. In the ascending portion the transverse diameter of the aorta measured 2.5 cm. From the arch, the innominate artery, the left common carotid and subclavian arteries arose. The patent ductus arteriosus continued into the right and left branches of the pulmonary artery. Below their division the pulmonary artery continued with a gradually decreasing lumen to the heart where it attached blindly as a fibrous band to the left of the aorta. No further anomaly was noted in the rest of the aorta. The left atrium showed nothing unusual; the foramen ovale was closed except for a narrow slit measuring 2 by 8 mm. The atrioventricular orifice measured 5 cm. in circumference; the valve appeared normal. The wall of the left ventricle measured 7 mm. in thickness. The trabeculae carneae and the muscoli papillares were well rounded. No opening was present at the usual site of the aortic orifice.

CASE 2. F. F., a 9 year old white boy, was brought in to the emergency room of the New Haven Hospital on June 7, 1933. He was dead upon arrival. The child had been under observation in the Outpatient Clinic for a congenital

heart condition. According to the parents he was one of eight children, the rest of whom were living and well. The child was born in 1923 by a normal spontaneous delivery at term after an uneventful pregnancy. He was a "blue baby."

When first seen in the Outpatient Clinic of the New Haven Hospital on July 29, 1926, the child appeared well developed and nourished. He became cyanotic when lying down and when crying. The heart was not enlarged to percussion, the rhythm was regular. A harsh systolic murmur, maximal in the third left interspace halfway between the sternum and the nipple, was audible over the whole precordium. A short systolic thrill was felt over the point of maximum intensity of the murmur. A diagnosis of congenital heart disease with interventricular septal defect was made. The patient returned to the Clinic on Feb. 13, 1928. During the interim he had developed difficulty in breathing, which became gasping. At times he was pale, at other times his lips and face were cyanotic. The left chest in the precordial region bulged slightly. The cardiac signs were the same as on the previous occasion. The pulse was 134 per minute and the temperature 39° C. His throat was injected and his tonsils were large. The next visit was on May 15, 1931. In the interval he had had increasing attacks of dyspnea and cyanosis upon exertion, which had necessitated a marked restriction of his activities. There was definite cyanosis of the ears, lips, tongue and extremities, and clubbing of the distal phalanges of the fingers and toes. The heart sounds were the same as on former occasions. Roentgenographic examination revealed no abnormalities in the size and shape of the heart. When seen again on Sept. 25, 1931, he had attacks of precordial pain, was markedly cyanotic and definite venous pulsation was noted. It was assumed that decompensation of the heart had commenced, and accordingly his activities were severely restricted. He was last seen in the Clinic on April 15, 1932, at which time he was deeply cyanotic. The systolic blood pressure was 94 and the diastolic 86 mm. Hg. On June 7, 1933, 30 minutes before being brought to the emergency room, he is said to have had an attack of dyspnea and cyanosis in which he stiffened out and cried for air. Five minutes before arrival he became limp and his breathing stopped. Both the cardiac pulsations and the respirations had ceased at the time of arrival.

At autopsy the body was well developed. A distinct bulging of the thorax was noted in the precordial region. There was a striking dusky cyanosis of the skin, mucous membranes and nail beds and a marked clubbing of the distal phalanges of the fingers and toes. All the viscera showed signs of marked chronic passive congestion.

Description of the Heart: The heart *in situ* appeared greatly enlarged. The rounded apex was composed equally of the right and left ventricles. The transverse diameter of the heart was 12.5 cm., while that of the thorax was 19.5 cm. There was a striking disproportion between the size of the aorta and pulmonary artery, the aorta having a diameter of about three times that of the pulmonary artery. The disposition of the inferior vena cava, the azygos vein and superior vena cava, the coronary sinus and its tributaries, appeared normal.

The right atrium was somewhat dilated. The atrioventricular orifice measured 11 cm. in circumference; the valve was thin and delicate, except for one firm translucent nodule 2 mm. in diameter situated on the line of closure of the anterior leaflet. The wall of the right ventricle measured 0.8 cm. The conus arteriosus was large and was directly continuous with the cavity of the right ventricle. At the base of the pulmonary valve it was 2.5 cm. in circumference. The pulmonary artery was small; it measured 1.5 cm. in circumference, as did also its right and left branches. The wall of the vessel was thin and presented a smooth, shiny intimal surface.

The pulmonary cusps were fused and markedly thickened. At the free margin the edges were rounded and beset with firm, pearly gray, roughened excrescences which measured 2 to 3 mm. in diameter. The fusion of the three cusps formed an inverted funnel with an aperture at the apex not more than 1 mm. in diameter. The left atrium was of usual size. The foramen ovale was closed. The atrioventricular orifice measured 7.5 cm. in circumference. The leaflets of the mitral valve were thin, delicate and well formed. The cavity of the left ventricle was increased in size and the columnae carnae were somewhat flattened. The wall measured 0.8 cm. in thickness. The myocardium was a homogeneous red-brown. At the base of the interventricular septum there was a circular defect, 1.5 cm. in diameter, involving chiefly the membranous portion. The defect began posteriorly at the middle portion of the posterior cusp of the aortic valve and ended anteriorly on a line with the junction of the right and left cusps. It was bounded below by the crescentic, rounded free edge of the interventricular septum. The aorta, a large thick-walled vessel, arose equally from both ventricles and was overriding the septal defect. The aortic orifice measured 5 cm. in circumference; its valves were thin and delicate. The right coronary artery arose from the right sinus of Valsalva, the left from the left sinus. The vessels pursued the usual course and distribution. The arch of the aorta was well formed and the vessels arose from it in the usual manner. Each of the two bronchial arteries noted measured 2 mm. in diameter. The ductus arteriosus was a thin fibrous cord connecting the pulmonary artery and the aorta.

Microscopic preparations of the pulmonary valve show the basal portion to be thin and composed of loose strands of connective tis-

sue. Toward the free margin it is thickened by numerous nodules of dense acellular hyalinized connective tissue. In several of these deposits of calcium are present. The connective tissue adjacent to the nodules is infiltrated by small and large mononuclear cells. Several thin-walled blood vessels are present in the valves adjoining the nodules.

The interstitial tissue of the myocardium is infiltrated with small mononuclear cells. In many regions these are grouped in small foci of 20 to 30 cells adjacent to blood vessels. In the right ventricle the cells are distributed diffusely throughout the myocardium. The myocardial fibers of this ventricle are increased in size and contain bizarre shaped nuclei.

DISCUSSION

The first case is one easily mistaken for a truncus arteriosus communis persistens. Two cases of this condition, which is rather rare, have been reported from this laboratory, one by Zimmerman² and the other by Finley.³ Recently, in an admirable review, Humphreys⁴ presented the criteria for identification of this malformation. In our case the surest landmark of the common trunk — the presence of four semilunar cusps, with two coronary arteries arising from the sinuses of opposite cusps — was absent. Furthermore, careful dissection disclosed the vestige of the pulmonary artery attached to the heart left of the aorta as a fibrous band. The latter, obtaining a gradually increasing lumen, continued into the right and left branches of the pulmonary artery; the patent ductus arteriosus connected these branches with the aorta. The case was thus identified as one of interventricular septal defect, atresia of the pulmonary artery and dextroposition of the aorta.

Stenosis of the pulmonary orifice without defect in the interventricular septum is, according to Abbott, "a lesion purely valvular and of inflammatory origin." When, however, the stenosis is associated with a defect in the interventricular septum, it is a malformation due to arrest in development. In our second case there is a healed valvulitis and a chronic diffuse myocarditis associated with this malformation. Thus, the question arises whether the stenosis of the pulmonary orifice is the result of the valvulitis or is a developmental defect with the valvulitis superimposed. The presence of

the septal defect favors the latter view. The rôle of the inflammatory process in the development of the stenosis cannot be estimated, although at present it is the most prominent feature.

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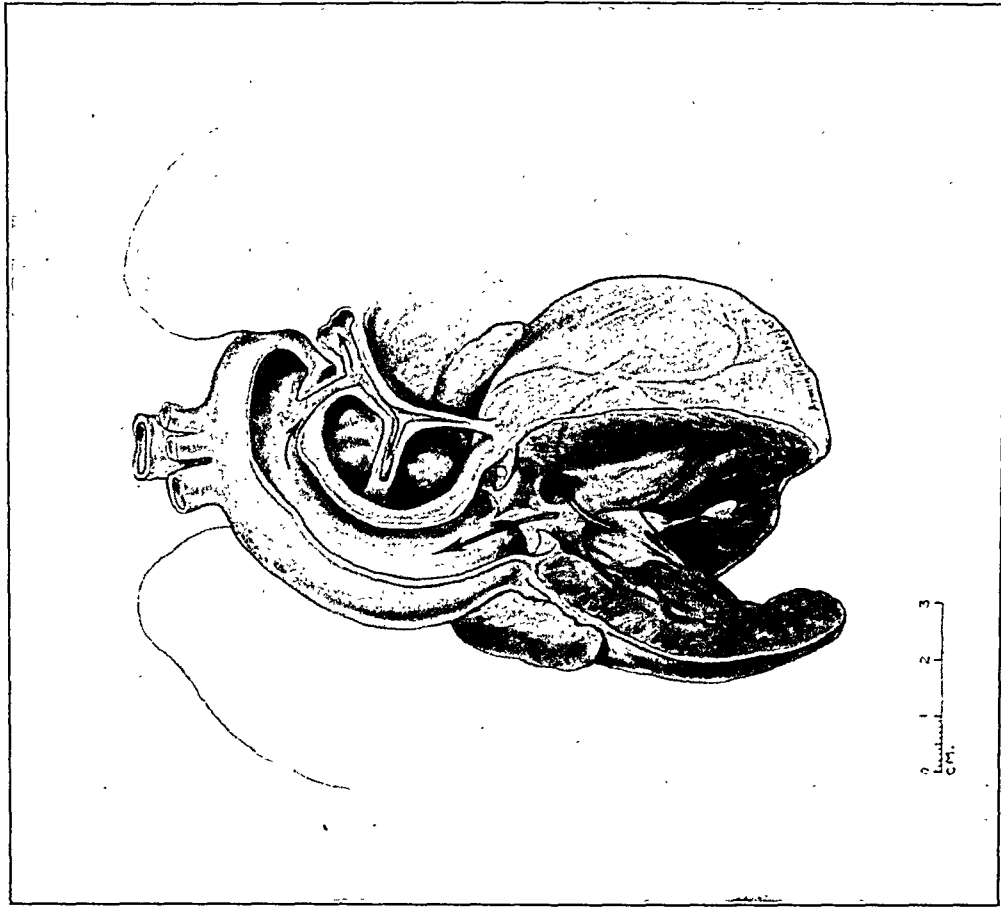
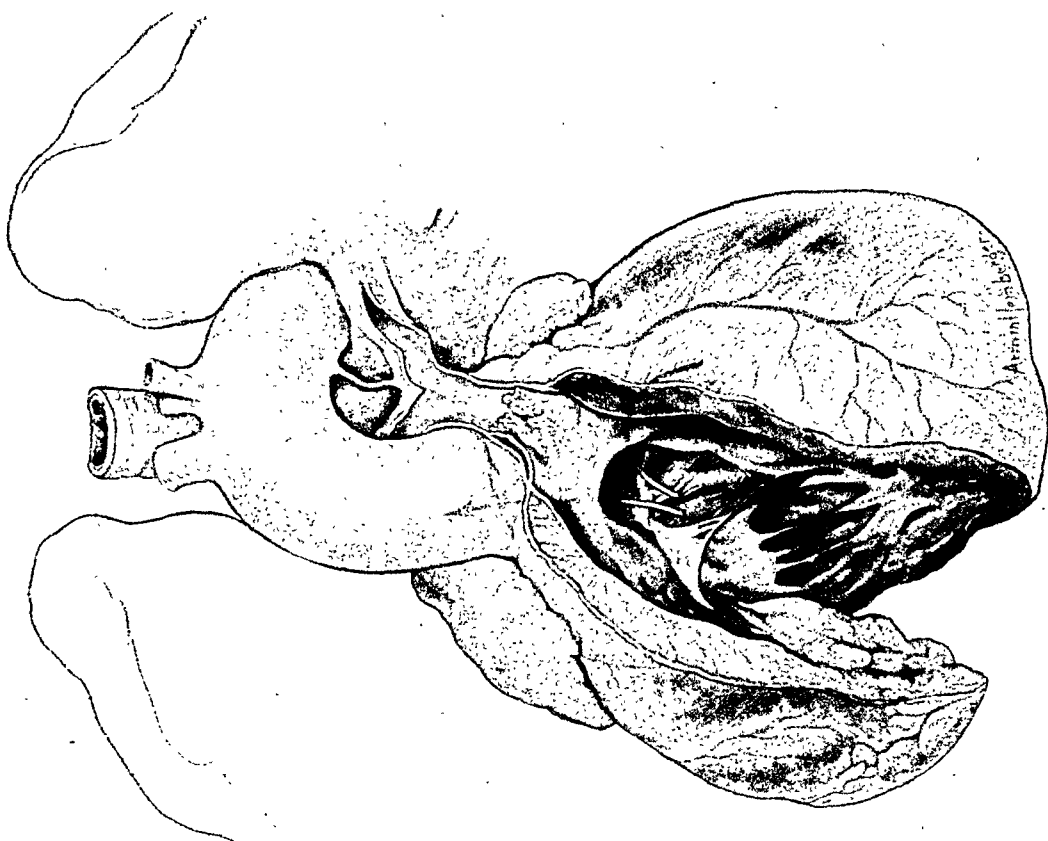
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DESCRIPTION OF PLATE

PLATE 99

FIG. 1. Interventricular septal defect, atresia of the pulmonary artery and dextroposition of the aorta in an 18 months old negro boy. From the conus arteriosus a single vessel, the aorta, ascends to form the arch from which the arteria anonyma, the left common carotid and subclavian arteries arise. Attached to the heart, left of the aorta, is a fibrous band which, obtaining a gradually increasing lumen, continues into the right and left branches of the pulmonary artery. The patent ductus arteriosus connects these branches with the aorta.

FIG. 2. Interventricular septal defect and stenosis of the pulmonary orifice in a 9 year old boy. The caliber of the pulmonary artery compared with that of the aorta is small. The semilunar valves are thickened and fused, narrowing the orifice.



THROMBOPENIC PURPURA ASSOCIATED WITH CARCINOMA OF THE STOMACH WITH EXTENSIVE METASTASES *

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Carcinoma is practically always associated with a normal or an increased number of platelets in the peripheral blood.^{1, 2} Perl³ has recently reported values at the lower limits of normal in the majority of the cases in her series. From time to time various investigators³⁻¹³ have reported exceptions to this general rule. Particularly uncommon is the association of thrombopenia with carcinoma of the stomach.^{4, 5, 7, 8, 11, 13} In addition to the cases in which there was a definite thrombopenia, Ellermann¹⁴ has described a case of carcinoma of the stomach with bone marrow metastases associated with ecchymoses. No estimation of the number of platelets in the peripheral blood was made by him. In the majority of the cases of carcinoma of the stomach with thrombopenia there have been extensive metastases to the bone marrow. Because of the rareness of the finding of thrombopenia as a complication of carcinoma of the stomach the present case, in which there were thrombopenia and extensive bone marrow metastases, is reported.

REPORT OF CASE

Clinical History: C. B., Unit No. 73002, 43 years of age, a male carpenter, was admitted to the Rochester Municipal Hospital on March 22, 1933, complaining of "stomach trouble." He stated that for 6 years he had had gaseous distention, belching of gas, sour eructations, and burning in the epigastrium and in the right abdomen. These symptoms occurred $\frac{1}{2}$ to 2 hours after eating and were relieved by vomiting or by soda, but not by food. He had vomited frequently and, on occasions, had noted food eaten 1 or 2 days previously in the vomitus. His appetite was poor. For 2 weeks he had been troubled with a severe constant pain in the lumbar region. This pain radiated to the hips, down the thighs and into the upper back. He had felt weak for 10 days. Three days before admission, and again on the following day, he vomited coffee ground material and passed semiliquid black stools. He had lost 30 pounds in weight in 4 years. He had noted pallor for several months, and also had noted that he

* Received for publication November 2, 1933.

bled easily after shaving. There was no history of petechiae, or epistaxis, and there had been no gross hematuria. The past history was unimportant. His father had had frequent severe epistaxis and had bled for 4 to 5 hours once following the extraction of a tooth.

Physical Examination: The patient appeared chronically ill. The skin and mucous membranes were pale. There was moderate epigastric tenderness associated with some muscle resistance. The liver was palpable just below the costal margin. No other findings of interest were observed on admission, but on the next day many petechiae were present on the lower extremities.

Laboratory Data: Table I shows the principal morphological blood findings during the period of observation. Repeated examinations of fixed blood smears stained with Wright's stain revealed slight achromia. The red blood cells showed slight variations in size, both microcytes and macrocytes being present and the average size being normal. No poikilocytosis was present. Occasionally nucleated red blood cells were found. In one preparation 8 normoblasts were seen while counting 200 white blood cells. The platelets were markedly diminished, averaging about 1 to 2 per oil immersion field. The coagulation time (test tube method) was 12 to 16 minutes. The clot retraction was good. The clot showed normal elasticity on one occasion and on two other occasions it was friable. The bleeding time varied between 10½ and 29 minutes. The icterus index was 6. Numerous urinalyses revealed no noteworthy abnormality. A small amount of albumin was present during the first week. After this the albuminuria remained about the same but casts were constantly present in varying numbers. On only one occasion was a rare red blood cell seen. Numerous clumps of white blood cells were found in one specimen. Repeated examinations of the stools showed the presence of blood during the first 12 days. No blood appeared after this time. The Wassermann reaction on the blood was negative.

Roentgenographs of the dorsal and lumbar vertebrae showed no metastatic lesions. A gastro-intestinal series revealed findings that were interpreted as carcinoma of the pyloric region of the stomach with high-grade obstruction.

Course in the Hospital: Two days after admission the patient vomited a large quantity of muddy, grayish material, which gave a strongly positive guaiac test. There was no further vomiting. Lumbosacral pain was one of the most troublesome symptoms, but it disappeared 20 days after he was first seen. The gastro-intestinal symptoms were partly alleviated by a Sippy diet. He was given large doses of iron in the form of Bland's pills and iron and ammonium citrate, without any effect on the blood picture. Twenty-five days after admission he suddenly became dyspneic and cyanotic in the morning and died that afternoon. Permission for autopsy was obtained.

POSTMORTEM EXAMINATION

External examination of the body and of the serous cavities reveals no findings of importance. The heart shows evidence of mild chronic tricuspid and subacute mitral endocarditis. The spleen, pancreas, adrenals, kidneys, pelvic organs, vascular system, neck organs and skeletal system show no noteworthy abnormalities.

TABLE I

Data on Blood Findings During Period of Observation

Date	Red blood cells	Hemoglobin	Reticulo- cytes	White blood cells	Differential count							Dege- nerated forms	
	per cmm.	gm. 100 cc.	per cent	per cmm.	Baso- philes	Eosin- ophiles	Myelo- cytes	Juveniles	"Stabs"	Seg- mented forms	Lymph- ocytes		Mono- cytes
3/22/33	3,500,000	9.0	...	10,700	0.5	2.0	69.0	19.5	7.0	2.0
3/23/33	3,680,000	9.2	1.1	11,700	0.5	71.0	15.0	12.0	1.5
3/26/33	2,650,000	7.0	...	8,400	8.0	78.0	10.0	4.0	...
3/28/33	2,500,000	6.0	...	6,500
3/29/33	2,770,000	6.8
3/30/33	2,660,000	6.2	...	6,200
4/2/33*	2,930,000	8.8	1.0	3.0	7.0	1.0	7.0	54.0	23.0	4.0	...
4/3/33	2,830,000	7.4	2.0
4/4/33	2,910,000	7.7
4/5/33*	3,400,000	8.2	...	10,550	11.0	3.0	11.0	54.0	16.0	5.0	...
4/6/33	3,200,000	7.7	1.6	6,100	...	0.5	7.0	69.5	17.0	6.0	...
4/7/33	1.9
4/8/33	3.6
4/10/33*	1.4	7,300	...	0.5	6.5	3.5	19.5	56.5	11.5	2.0	...
4/11/33	2,900,000	7.9	1.7	6,000	1.0	...	15.0	65.0	14.0	5.0	...
4/12/33	2.1
4/13/33	1.7
4/14/33*	8,950	1.0	1.0	7.0	4.0	17.0	52.0	14.0	2.0	2.0

* Schilling differential counts done at this time. In all other counts all neutrophils were classified as segmented forms.

Lungs: The left lung weighs 625 gm., the right lung 700 gm. The pleural surface of the right lung is covered with fibrous adhesions, that of the left is smooth and glistening. They are mottled red and black, voluminous and somewhat heavy, crepitant to spongy in consistence, and along the margins are distended alveoli. The hilum nodes are small and deeply pigmented. The bronchial mucosa is bright red and injected. The pulmonary vessels are elastic and show no thickening. At the apex of each lung is a small puckered scar. Sections through the lungs reveal bright red, glistening surfaces from which fluid can easily be expressed. There is no evidence of consolidation.

Gastro-Intestinal Tract: The mucosa of the esophagus is smooth and velvety. The stomach contains about 500 cc. of light brown, fluid material, and at the pyloric region is a firm, annular mass, involving the walls for a distance of about 2 cm. above the ring. In one area this mass is ulcerated and presents a gray, necrotic crater. Section through the mass reveals a firm, white, fibrous, glistening surface. This tissue has invaded the peritoneum about the pylorus and several firm white lymph nodes of the same consistence are noted in this region.

Liver: Weight 2150 gm. The organ is large, dark brown, and over the surface of all lobes are small, rounded gray areas which are depressed below the surface. These vary in size from 2 mm. to 1 cm. in diameter, and are also visible on cut section. Many of them show small areas of hemorrhage and a few are surrounded by a hyperemic zone. The remaining liver tissue appears normal. The lobulation is regular and distinct.

Gall-Bladder: About 50 cc. of viscid bile is present in the gall-bladder. The wall is not thickened and the mucosa is smooth, the ducts patent.

Lymphatic System: In the retroperitoneal region are several large grayish glands which are firm and on cut section present a uniform gray surface.

Bone Marrow: The shaft in the region of the middle third of the femur appears thickened. The marrow is quite firm and on pressure holds its shape. Bone trabeculae are conspicuous in the marrow cavity. There is only a slight amount of the red element present, the greater portion being fat. The marrow of the vertebrae is red and appears somewhat hyperplastic. Section of the rib shows the

marrow cavity to be nearly empty, showing no hyperplasia. The marrow cavity of the sternum contains many bone trabeculae. The marrow here is also firm and shows only slight hyperplasia.

Anatomical Diagnoses: Carcinoma of the stomach with metastases to the liver, lungs, adrenals, bone marrow, and lymph glands; pulmonary congestion and edema; acute bronchitis; subacute mitral and chronic tricuspid endocarditis; chronic cystitis; emphysema; healed apical tuberculosis; chronic pleuritis.

MICROSCOPIC EXAMINATION

Heart: The cardiac fibers appear small and there is a small amount of yellow pigment at the nuclear poles. Sections show no scars.

Lungs: The alveolar walls are congested and in many areas their lumens are filled with fibrin and fluid. Large endothelial phagocytes and heart failure cells are conspicuous. There are several small areas of infarction showing uniform infiltration with red cells and destruction of alveolar architecture. The most striking feature is the distribution of tumor cells. The lymphatics about the bronchi and vessels, and the capillaries of the alveolar walls, are in most areas filled with tumor cells. Also, the smaller vessels and the lymphatics of the pleura show a similar condition. The cells are large with irregular, pale cytoplasm. The nuclei are round or oval with prominent mitoses. There are no areas in which nodules of tumor have eroded lung tissue. A few small vessels contain thrombi, apparently invaded by tumor. There is no increase in fibrous tissue.

Spleen: The pulp is considerably congested. The malpighian bodies are small and there is conspicuous thickening of the central vessels. No tumor cells are present in the section.

Gastro-Intestinal Tract: Sections of stomach show epithelium that has undergone postmortem change and is eroded. In one portion it becomes atypical, presents a diffuse distribution and the cells are heaped up. The wall in this portion is thick and the muscle is almost entirely replaced by connective tissue. Tumor cells have invaded this area but are in small clusters and infrequent. They are surrounded in all instances by very dense fibrous tissue. The cells have round or oval, deep staining nuclei, rich in mitoses, and are quite small. Tumor cells are present on the serous surface, and there is evidence of chronic inflammation, with many clusters of round

cells. In one area the mucosa is definitely eroded, infiltrated with neutrophils and covered with cellular débris.

Liver: There are clusters of tumor cells that have stimulated a marked growth of connective tissue, almost completely obliterating the normal architecture. In these areas the proportion of connective tissue is far greater than that of tumor. The surrounding liver cells are atrophic and contain yellow pigment. Throughout the entire section there are tumor cells within the sinuses and in many lobules, completely filling the central veins. The cells are similar to those noted in the lungs and stomach. This latter type of tumor infiltration has produced no connective tissue formation. The distribution is remarkably like that seen in the leukemias. Many neutrophils are seen throughout the sinuses.

Adrenals: There is invasion of the medulla with tumor cells. They have not entirely destroyed the medullary tissue, are not abundant, and have stimulated no connective tissue formation.

Kidneys: A few small scars are present beneath the capsule, infiltrated with round cells and involving hyalinized glomeruli. The tubular epithelium is somewhat swollen and granular.

Bladder: The mucosa is eroded, and the submucosa is somewhat thickened and infiltrated with round cells. The muscle fibers are enlarged.

Lymphatic System: The mesenteric glands are almost entirely replaced by tumor cells.

Thyroid: Shows the usual adenomatous change. There is a slight amount of colloid in the acinar lumens.

Bone Marrow: The femoral marrow cavity shows a large amount of fibrosis. The connective tissue is quite dense and cellular, and spicules of bone are prominent. They are dense and show no evidence of destruction by osteoclasts. Throughout the marrow cavity are clusters of tumor cells, similar to those described in the stomach, which lie between the bony spicules and have not eroded the bone. A section through the shaft of the femur shows dense bone formation with clusters of eosinophilic and neutrophilic myelocytes, as well as the mature polymorphonuclear type. Erythroblasts and mature red cells are conspicuous. Normal appearing megakaryocytes are present but are probably diminished in total numbers. The sternal marrow shows a similar picture but the fibrosis is more marked and fewer tumor cells are noted. There is much less evidence of blood

cell formation in this region and the bony spicules are more prominent. Section of the vertebra shows an extensive infiltration of tumor cells without erosion of bone. There is moderate hyperplasia, possibly more marked than in the femur, of both red and white cell progenitors. Normal appearing megakaryocytes are numerous. Figure 1 shows a typical section of the vertebral marrow. Clusters of tumor cells are interspersed with normal bone marrow.

DISCUSSION

So far as the blood picture is concerned, there is only one finding that is unusual — the marked diminution in the number of the platelets. The exact mechanism responsible for this cannot be given. It would seem that the presence of large numbers of tumor cells in the bone marrow was the probable cause of the thrombopenia. However, studies of the bone marrow sections reveal an approximately normal number of morphologically normal megakaryocytes. Nevertheless, the presence of these megakaryocytes does not prove that these cells were functionally normal. Increased peripheral destruction or loss of platelets cannot be excluded.

The question naturally arises as to the relation of the thrombopenia to the hemorrhagic diathesis. It seems to us that the connection is most probably a close one, although it would be impossible to say that the bleeding phenomena were not dependent, at least in part, on changes that may have occurred in the permeability of the capillary walls, as is thought by many to be the case in idiopathic thrombopenic purpura.

The presence of large numbers of early cells of the myeloid series and of numerous nucleated red blood cells is not unusual, and has been commented upon previously.^{2, 4, 5, 6, 10, 11} These findings should be emphasized, however, as they may aid in differentiating idiopathic thrombopenic purpura from the symptomatic form in cases where the presence of a malignant tumor is not proved. It is unusual to find large numbers of nucleated red blood cells and many early myeloid cells in idiopathic thrombopenic purpura, although they may be found after recent massive hemorrhage.

The value of studies of the sternal marrow in such individuals cannot be too strongly emphasized. Had we been able to obtain a sternal marrow biopsy from this patient during life, a positive

diagnosis could have been made. This procedure was planned for this patient but his sudden death prevented its being done.

SUMMARY

The clinical and postmortem findings in a patient who had thrombopenia associated with carcinoma of the stomach with extensive metastases are reported.

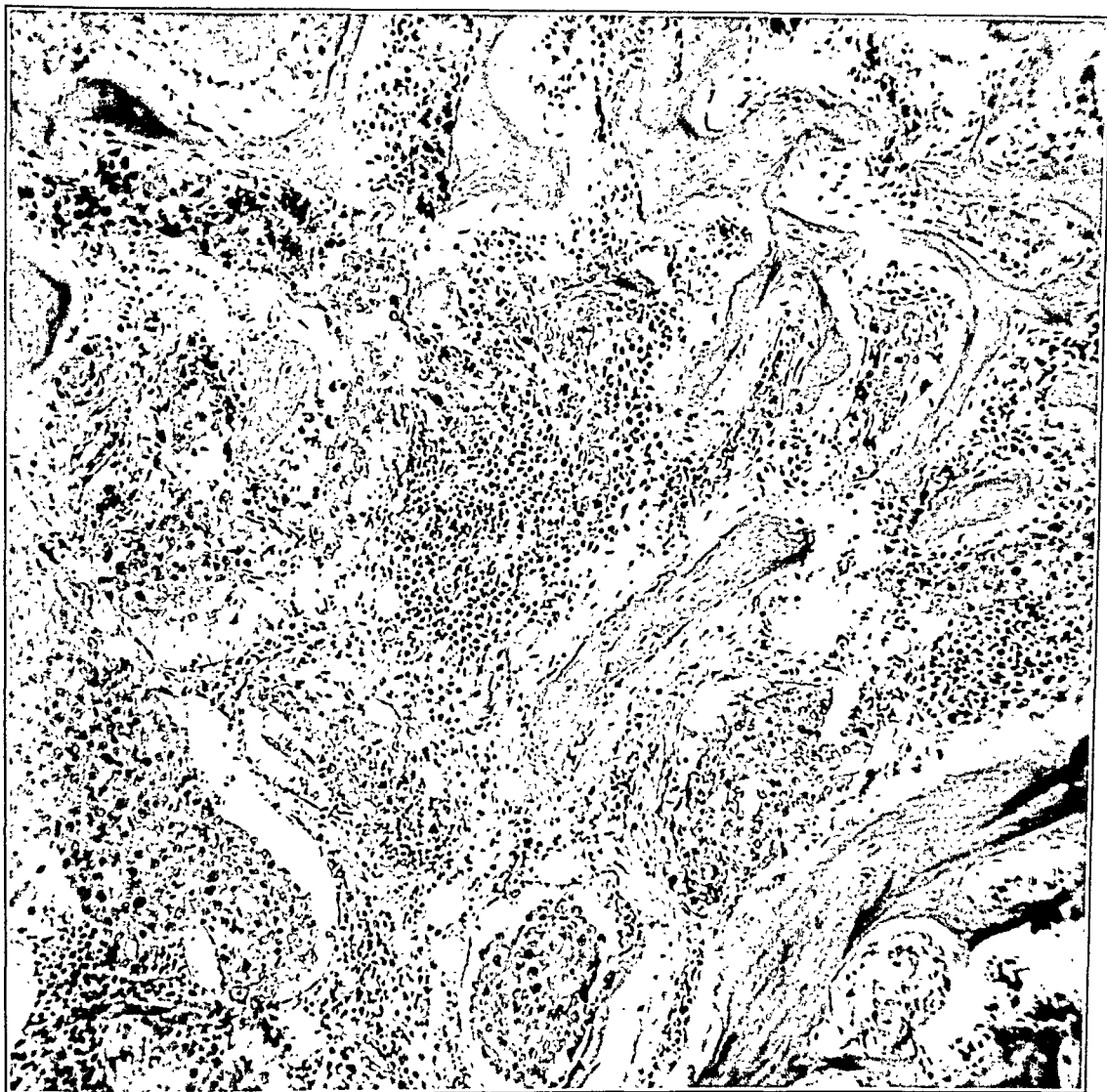
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DESCRIPTION OF PLATE

PLATE 100

FIG. 1. A typical section from the vertebral marrow. Note the presence of clusters of tumor cells interspersed in normal appearing bone marrow. $\times 115$.



I

TRACHEO-ESOPHAGEAL FISTULA OF SYPHILITIC ORIGIN *

REPORT OF A CASE

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Syphilis, as the etiological factor of tracheo-esophageal fistula, is uncommon. In 1899 Sirot¹ collected 143 cases, of which 68 were due to neoplasm, 11 to foreign bodies, 2 to pressure of a tracheal cannula, and 4 of which followed a cicatrix, 17 inflammatory lesions such as tuberculosis, 3 perforation of an ulcer, and 1 each to aneu-

TABLE I

Data on Instances of Tracheo-Esophageal Fistula of Syphilitic Origin Collected from the Literature

Author	Sex	Age	Site of tracheal lesion
		yrs.	
Berger (quoted by Conner ³)	M	33	Upper 1/3
von Navratil ⁹	M	28	Upper 1/3
Krassnigg ¹⁰	M	53	Middle 1/3
Schütze ¹¹	F	42	Lower 1/3
Gerber ¹²	M	65	Lower 1/3
Levy ¹³	F	24	Lower 1/3
Sonntag ¹⁴	M	34	Lower 1/3
Beeler ⁴	M	58	Lower 1/3
Dufour ¹⁵	F	38	Not given
Moritz ²	F	36	Not given
Basch ¹⁶	Not given
Curschman (quoted by von Fraenkel ⁸)	F	41	Not given

rysm and trophic disturbances. In 36 cases the cause was undetermined. Only 1 of these, a case reported by Moritz,² was of definite syphilitic origin. In 1903 Conner³ reported 128 instances of syphilis of the tracheobronchial tree. Two of these tracheal ulcers were followed by a perforation into the esophagus. We were able to collect only 12 instances of tracheo-esophageal fistula of syphilitic origin from the literature, and in 1 of these, Beeler's case,⁴

* Received for publication November 4, 1933.

there is some doubt as to the correctness of the diagnosis. The author himself raises the question. Table I is a brief résumé of the 12 collected cases. The case which follows is an addition to that list.

REPORT OF CASE

Clinical History: The patient, a negress aged 42 years, was admitted to the Jefferson Medical College Hospital on Aug. 15, 1932. For 10 days she had had great difficulty in swallowing both solid and liquid food. This alarming symptom developed very suddenly and was associated with a violent paroxysm of coughing and considerable expectoration. Prior to this event, the patient had had some cough but no expectoration. For some time she had noticed some dyspnea which became progressively worse. The dysphagia had been so intense that not only the swallowing of solids and liquids, but also attempts to swallow saliva induced a violent fit of coughing and choking. Expectoration was profuse, purulent, foul smelling, bloody, and at times contained foodstuffs such as milk, which was readily recognized. There was no pain in the throat or chest. Since the onset of illness the patient had lost weight rapidly.

The previous medical history contained little of interest. The patient had had five children. There was no history of miscarriages. She had had three consorts. The personal and familial record of tuberculosis and malignant disease was negative. There was no history of swallowing escharotics or of the presence of a foreign body in the trachea or esophagus.

Physical Examination: The patient was an extremely emaciated adult negress. The face was thin and pinched, the eyes sunken, the lips dry and parched. The skin was dry and harsh. There was remarkable freedom from dyspnea when the patient was quiet. She was not hoarse.

The eyes reacted to light and accommodation and the pupils were equal.

The mouth was rather dirty. Many of the teeth were decayed; the gums and gingival margins were the seat of a low grade, suppurative process. The tongue was markedly coated. The tonsils were not diseased and there were no paralyses affecting the tongue or soft palate.

The mucosa of the larynx was pale. Motility of the arytenoid cartilage and the vocal cords was not impaired. The pyriform sinus contained a small amount of saliva. No gross lesions were found in the larynx. Examination of the neck was essentially negative. The chest was long and flat and because the patient had lost so much weight, the ribs stood out prominently. There was no asymmetry of the thorax and the expansion was equal on both sides. Vocal resonance and tactile fremitus were not abnormal and percussion elicited no areas of impairment. Breath sounds were vesicular in character and moist râles were present at both bases of the lungs.

The heart was not enlarged and auscultation elicited no adventitious sounds.

The abdomen was rather markedly distended, the distention being chiefly confined to the upper half. The liver and spleen were not palpable. No pain, tenderness, rigidity or signs of fluid were present.

An examination of the extremities, including the testing of the reflexes, revealed nothing of importance.

The superficial lymph nodes, including the epitrochlear nodes, were palpable, small and hard.

The temperature was 99 F, the pulse rate 140 and the respiratory rate 28 per minute, on admission.

Special tests were made to determine the nature of the disability in swallowing. The patient was placed successively in the recumbent position, sitting position, on the right side and left side, and lying on the abdomen. While in each of these positions she was given a few sips of water to swallow. In the recumbent position she was able to swallow the water without difficulty. In the other positions the act of swallowing was accompanied by a violent paroxysm of coughing and expectoration of the swallowed fluid. A very brief but definite time intervened between the swallowing of the water and the onset of the cough.

The roentgenological report is as follows: "There is no evidence of a pulmonary lesion. The heart and diaphragm shadows are normal. We were unable to get films of the patient's swallowing function because every time we had her drink barium she had to cough. The barium gets down the pharynx, but instead of going down the esophagus, seems to be displaced so that it passes into the trachea, causing the patient to cough. However, I believe a very small amount of barium did get into the stomach. The area of obstruction seems to be at the beginning of the esophagus."

The laboratory reported a 4+ reaction of the blood when tested by the Wassermann and Kahn methods. The blood count was as follows: hemoglobin 65 per cent, erythrocyte count 4,000,000, and leukocyte count 14,800. The sputum was repeatedly examined for tubercle bacilli but none was found. The results of other laboratory tests were unimportant.

Esophagoscopy was performed and the report is as follows: "The pus was traced down to an opening in the esophagus which is partially covered by a necrotic flap. It is situated 21 cm. from the upper teeth, in the left quadrant of the esophagus. Air was forced through this fistula at each expiration and the necrotic flap blew in and out with the air current. The fistula is approximately 2 cm. in diameter. A Rehfuess tube was inserted into the stomach by the aid of the esophagoscope for feeding purposes." (See Fig. 1.)

The patient was given antiluetic treatment but became progressively worse. The temperature mounted to 105° F and symptoms of bronchopneumonia supervened. Death took place 5 days after admission.

POSTMORTEM EXAMINATION

The body is that of an extremely emaciated adult colored female, aged 42 years. The lips are dry, the tongue coated, the teeth partially decayed and about the gums there is some pyorrhea. Superficial lymph nodes of the neck, axilla, groin and epitrochlears are small and hard.

Pleural, pericardial and peritoneal membranes are smooth and glistening and the respective cavities contain no excess of fluid. There is a mass filling the entire superior mediastinum. The thoracic contents are removed *en masse*.

Superior Mediastinum and Contents: The lymph nodes of the superior mediastinum are enlarged and firm, and are embedded in a

firm fibrous mass that also binds the other structures, vessels, trachea and esophagus together. On section the lymph nodes are yellowish white, firm, homogeneous, slightly granular and opaque. On the inferior aspect of the arch of the aorta there is a small aneurysmal pouch 1 cm. in depth. The endothelium of the vessel in this pouch is scarred, puckered and white, making a distinct contrast beside the yellow color of the intima of the aorta.

Trachea: The trachea is adherent to the esophagus and the other structures of the mediastinum. On section a large, circular, ragged ulcer measuring 6 cm. in its greatest diameter is found (Fig. 2). This ulcer is on the left posteriolateral aspect, reaching from the level of the first to the eighth tracheal rings. The margins of the ulcer are irregular, and slope down to the base in a step-like manner. The floor of the ulcer is ragged, dull, green and friable. The tracheal cartilages in the ulcerated area have completely sloughed and there is no vestige of them remaining. In the center of the ulcer there is a perforation 2 cm. in diameter, which communicates with the esophagus. The tracheal side of the perforation is surrounded by ragged friable shreds of tissue. The firm adhesions between the two organs have prevented leakage of contents into the mediastinal space.

Esophagus: At the upper end of the esophagus there is an oval-shaped perforation 2 cm. in diameter which communicates with the trachea (Fig. 3). The margin of the stoma is slightly elevated, fairly firm, quite red and clean cut. The remainder of the esophagus presents no gross pathological lesions.

Lungs: Both lungs are edematous and throughout there are numerous, small, red, firm areas of consolidation that fade imperceptibly into the sound tissue. The mucosa of the bronchi is swollen and red and the bronchi are filled with yellow purulent material. No gross lesions of tuberculosis are present.

Heart: Weight 470 gm., measurements 15 by 9 by 6 cm. The organ is of moderate size and there is slight hypertrophy of the left ventricle, otherwise it has no demonstrable pathological lesions. The aorta has no lesions of consequence except the small aneurysm described above.

Diaphragm: This appears to be without pathological change.

Spleen: Weight 150 gm., measurements 14 by 8 by 3 cm. No gross lesions are found, but considerable blood oozes from the cut surface.

Liver: Weight 1850 gm., measurements 28 by 24 by 7 cm. This organ is fairly large, flabby and chamois-colored. On section the parenchyma sinks beneath the cut edge and blood oozes from the cut surface. The left lobe gives the impression of being larger than usual. In the lower anterior portion there is a sharply circumscribed, irregularly shaped tumor measuring 7 cm. in diameter (Fig. 4). Its surface protrudes slightly above that of the liver and the mesentery is adherent to it. The tumor is pale yellow, firm and nodular. On section it is pale yellow, firm, homogeneous, dull, opaque and slightly granular. At its center there is some necrosis with softening.

Kidneys: The right kidney weighs 150 gm. and measures 11 by 6 by 4 cm.; the left weighs 120 gm. and measures 10 by 6 by 4 cm. They are somewhat red and on section considerable dark red blood oozes from the cut surface.

The following organs show no gross pathological lesions: *gall-bladder and biliary ducts, adrenals, pancreas, ureters, bladder, stomach, small and large intestine, and the abdominal lymph nodes.*

Uterus: A few small, subserous myomas are present which measure about 0.5 cm. in diameter. The *ovaries* are fibrosed. The *fallopian tubes* are dilated, tortuous and adherent to the ovaries. They contain no pus or other fluid.

MICROSCOPIC EXAMINATION

The lesions that are of particular interest histologically are those of the trachea, thoracic lymph nodes and liver. Microscopically the ulcer of the trachea consists of a central necrotic area and a marginal cellular zone. In the marginal zone there is a decided increase in fibrous tissue and capillaries. The tissue, particularly about the vessels, is infiltrated with cells of inflammatory origin, with a preponderance of fibroblasts, epithelioid cells, lymphocytes and other mononuclear cells. In addition to these there are many plasma cells, polymorphonuclear leukocytes, eosinophiles and a few giant cells.

The lymph nodes have a lesion histologically similar to that of the tracheal ulcer. In addition the structure of many of the nodes is completely replaced by fibrous tissue. There is also some deposition of carbon particles present.

The tumor of the liver has the microscopic structure of a granuloma. There is a central area of necrosis and a marginal zone of in-

flammatory reaction. At the periphery of the lesion the fibrous tissue is greatly increased and is compressing the liver cells, which are atrophied. Here and there in the tissue the bile ducts have proliferated. At the very margin of the necrotic area there are many newly formed capillaries which are filled with blood. About these and in the tissue adjacent to the necrosis are numerous cells of inflammatory origin. These are fibroblasts, epithelioid cells, lymphocytes, mononuclear cells, polymorphonuclear leukocytes and a few plasma cells. Giant cells are present but few in number. Sections from all of these tissues were stained for tubercle bacilli but none was found. Preparations were made to demonstrate *Treponema pallidum*, for which a modification of Warthin's technique⁵ was employed. None was demonstrated (Fig. 5).

The histological studies of the lesions of the other organs are not of sufficiently unusual character or importance to detail their description here. The following gross and microscopic anatomical diagnoses were made.

General Diagnoses: Tertiary syphilis; gumma of the trachea; tracheo-esophageal fistula (syphilitic); chronic syphilitic lymphadenitis; gumma of the mediastinal lymph nodes; early aneurysm of the aorta; bronchopneumonia; acute suppurative bronchitis; myocardial fibrosis; gumma of the liver; passive congestion of the liver, spleen and kidneys; subserous fibromyoma of the uterus, and chronic salpingo-oöphoritis (non-tuberculous).

DISCUSSION

The syphilitic nature of the lesion is established, we believe, beyond reasonable doubt. The strongly positive Wassermann and Kahn tests, the concomitant gumma of the liver, incipient aortic aneurysm, gummas of the lymph nodes, absence of tuberculous lesions in the lung or elsewhere, and the failure to find tubercle bacilli in repeated sputum examinations or in the sections of the tissue lesion, make up the evidence upon which the diagnosis rests. In addition, the histological character of the lesions is of great importance. The rather marked increase of fibrous tissue, the vascularity of the lesions, scarcity of giant cells and general lack of orderliness in the marginal cellular area, differentiate it fairly well from the tubercle. Like MacCallum⁶ we are not convinced that a few giant

cells in these gummatous lesions mean an associated tuberculous lesion, as Baumgarten ⁷ believes.

The combination of tracheo-esophageal fistula, gummas of the liver and lymph nodes, and an incipient aneurysm is quite extraordinary. As far as we have been able to determine from the literature this case is the only one with so many concomitant syphilitic lesions. Von Fraenkel ⁸ quotes a case reported by Curschman in which gumma of the liver was an associated lesion. Berger, as quoted by Conner,³ reports an instance in which gumma of the testicle was also present. Up to the time of death the gumma of the trachea and the subsequent fistula were the only lesions that produced symptoms. The lesion of the liver and the aneurysm were discovered at autopsy.

SUMMARY

A case of tracheo-esophageal fistula of syphilitic origin is reported. Other lesions present were gummas of the liver and lymph nodes and incipient aneurysm of the aorta.

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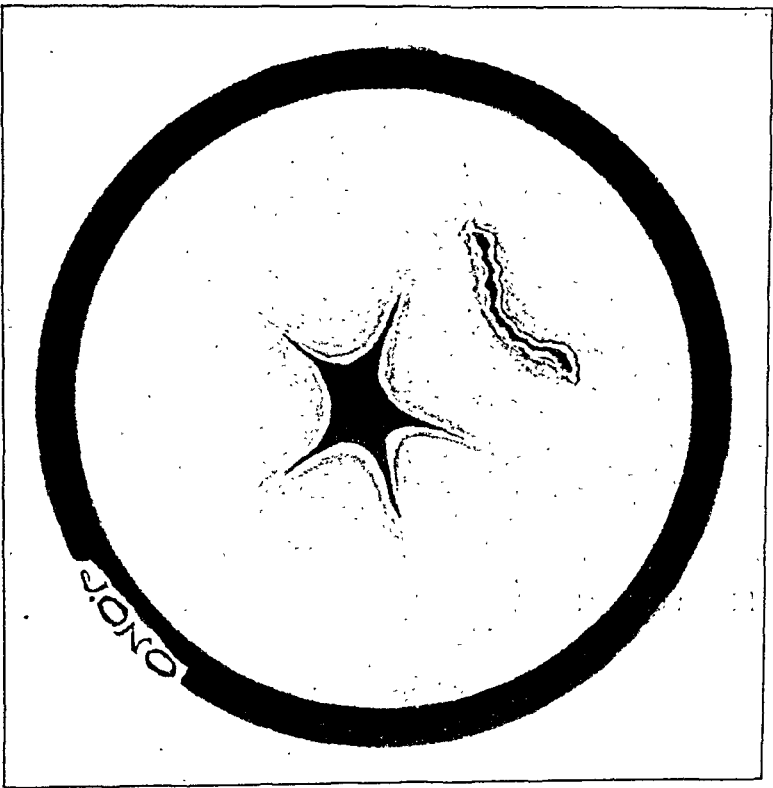
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DESCRIPTION OF PLATES

PLATE IOI

FIG. 1. Esophagoscopic appearance of the fistula.

FIG. 2. Syphilitic ulceration of the trachea.



I



21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100

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PLATE 102

FIG. 3. Stoma of the tracheo-esophageal fistula in the esophagus.



PLATE 103

FIG. 4. Gumma of the liver.

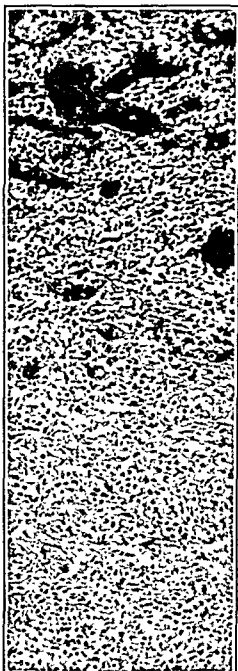
FIG. 5. (*a*) Microscopic section of the gumma of the liver. (*b*) Microscopic section of the tracheal ulcer. (*c*) Microscopic section of the thoracic lymph node.



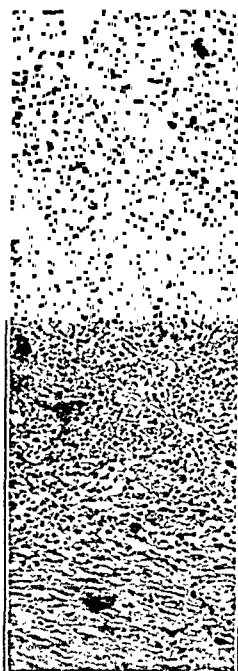
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5a



5b



5c

MYXOMA OF THE HEART VALVES *

REPORT OF A CASE

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Although all tumors of the heart are rare, primary tumors of the heart do occur in the form of either sarcoma, fibroma, lipoma, myoma or myxoma, or combinations of two or more of these. With the exception of sarcoma these tumors tend to be benign. There has been considerable confusion and controversy about the classification of myxoma and fibroma of the heart ever since Czapek ¹ asserted that many of the alleged myxomas of the heart were really organized thrombi. Thorel,² Stahr,³ and Husten ⁴ concurred with Czapek in this opinion, while Ribbert,⁵ Link,⁶ Mandelstamm,⁷ and Hagedorn ⁸ presented strong arguments in favor of the neoplastic nature of these tumors. The differentiation is made difficult by the microscopic similarity between myxoma and organized thrombi. Both show a homogeneous, poorly cellular matrix, and both are covered with endothelium. They differ, however, in that the myxomas are avascular, and usually have mucin and elastic fibers present, while organized thrombi are vascular, generally contain degenerated blood pigment, and have neither mucin nor elastic fibers. When these differences are considered, along with the gross appearance, there is little room for argument, for the organized thrombus is typically a smooth flat tumor with a broad base and more or less evidence of contracture and scarring, while the myxoma is a pedunculated, lobulated tumor with a narrow base and no evidence of contracture.

Although myxomas have been found in all chambers of the heart the sites of predilection are the interauricular septum of the left auricle and the heart valves. The myxomas that are found on the heart wall and septum are characteristically large tumors that may grow to a size sufficient to fill the entire chamber in which they are located and cause valvular insufficiency by occlusion of the valves.

* Received for publication November 15, 1933.

Choisy⁹ first described a heart polyp of the left auricle situated in the valvula foraminis ovalis. Within the next few years similar cases were reported by de Puisaye,¹⁰ LeGendre,¹¹ and Proust.¹² These tumors had the lobulated appearance and microscopic peculiarities of the myxoma. The valvular myxomas are more uncommon than those in the heart wall and are much smaller, varying from 6 to 15 mm. in diameter. They resemble the larger growths in that they are pedunculated, but show a characteristic papillary structure which the larger ones do not have. They are found on all the heart valves but are slightly more common on the tricuspid valve.

A total of 22 myxomas located on the heart valves have been reported. These are tabulated in the accompanying table. A consideration of the table will show that these tumors may occur at any age from birth to old age, and in either sex. As a rule, they are found accidentally at autopsy, having failed to give rise to clinical signs or symptoms during life.

REPORT OF CASE

Clinical History: A. H., a negress, 62 years of age, was admitted to St. Luke's Hospital on Dec. 26, 1932 because of failing vision in both eyes. Except for a pelvic operation 8 years before she had always been well.

The patient was an obese colored female with normal vital signs and, except for the eyes, normal physical findings. The pupils were equal, but both lenses showed large opacities. After a preliminary iridectomy the patient was discharged and readmitted 3 weeks later for a right cataract extraction, which was uneventful. On the 6th postoperative day she suddenly collapsed and showed the typical picture of circulatory failure, the blood pressure being 60/40 and the pulse feeble. Roentgenograms and electrocardiograms offered no assistance in making a diagnosis. She became steadily worse and died the next day.

POSTMORTEM EXAMINATION

The body was that of an obese colored female. The positive findings were limited to the heart and pericardial sac. On opening the pericardial sac about 100 cc. of cloudy fluid were seen. The pericardium was hemorrhagic, and covered with small tags of fibrin. The heart weighed 325 gm. and the left ventricle measured 1.5 cm. in thickness. None of the valves was thickened, and there was no evidence of endocarditis. On the anterior cusp of the tricuspid valve, situated in the middle of the valve and about 5 mm. from its

TABLE I
Summary of Previously Reported Valvular Myxomas

Author	Year	Type of Tumor	Situation	Age yrs.	Sex	Remarks
Luschka ¹³	1857	Myxoma?	Pulmonic	40	F	Old endocarditis present. Author considered it inflammatory
Curtis ¹⁴	1871	Myxoma	Mitral	83	F	
Debove ¹⁵	1873	Myxoma	Tricuspid	38	M	All were highly papillary
Ribbert ¹⁵	1897	Myxoma	Pulmonic	"	"	
			Tricuspid	"	"	
			"	"	"	
Guth ¹⁶	1898	Papillary myxoma	Tricuspid	54	F	Other valves normal
Simmonds ¹⁷	1901	Papillary fibroma	Tricuspid	25	..	Endocardium thickened. Multiple fibromas in walls
Reitmann ¹⁸	1905	Hyalofibroma	Pulmonic	74	M	Papillary; matrix very poor in cells
Leonhardt ¹⁹	1905	Myxoma	Mitral	26	F	Valve thickened
Djewitsky ²⁰	1906	Myxoma	Aortic	38	M	
Blumgart ²¹	1907	Fibromyxoma	Mitral	86	F	
Hagedorn ⁸	1908	Myxoma	Mitral	Middle aged	M	Patient had a hypernephroma
Koechlin ²²	1908	Myxoma	Pulmonic	19	F	Koechlin considered these tumors to be Lambd excrescences
			"	53	"	
			Aortic	60	"	
Forel ²³	1910	Fibroma	Aortic			
Dean and Falconer ²⁴	1913	Myxoma	Pulmonic	53	M	Some thickening of cusp
Kornfeld ²⁵	1928	Myxoma	Pulmonic	68	M	
Abrahamer ²⁶	1931	Myxoma	Pulmonic	Newborn infant		Similar growth on tricuspid
			Aortic	"		

edge, was a small spherical tumor measuring 6 mm. in diameter and projecting 4 mm. above the surface of the valve, to which it was attached by a short wide pedicle. Its surface was finely nodular and it had a translucent gelatinous appearance (Fig. 1). The coronaries were thin and showed no evidence of sclerosis.

Permission for examination of the brain was not obtained, but it was assumed that the patient died of a cerebral accident.

MICROSCOPIC EXAMINATION

The cusp containing the tumor was excised, and sections were prepared with hematoxylin and eosin, Van Gieson's, thionin, mucicarmine, and Weigert's elastic tissue stains.

There is no microscopic thickening of the cusp, and there is well marked differentiation of the layers of the valve. A narrow pedicle attaches the tumor to the cusp, and the endothelium and elastic laminae are reflected onto the pedicle. The pedicle very shortly spreads out, and divides into two main limbs, from each of which numerous papilliform processes arise (Fig. 2).

Stained with hematoxylin and eosin the tumor matrix is pink. There is a complete endothelial covering over the entire tumor. The papillae contain only a few cells and there are no blood vessels present; the pedicle, on the other hand, contains many stellate and spindle-shaped cells which have fine processes extending from them into the fine fibrillar groundwork present throughout the entire tumor (Fig. 3).

The test for mucin with thionin is only faintly positive; the stain with mucicarmine, however, is strongly positive, the entire matrix taking a deep red stain. Van Gieson's connective tissue stain shows a large number of coarse, pink-staining fibrils in the pedicle which extend into the papillae and become lost. The presence of elastic fibers is proved by Weigert's elastic tissue stain, which shows a large number of vertically placed, blue-staining fibers throughout the entire tumor.

The tumor presented then is one arising by a well circumscribed pedicle from the tricuspid valve, and which is free of any inflammatory change. The tumor contains mucin and elastic fibers. Part of this tumor, however, consists of hyaline connective tissue.

DISCUSSION AND SUMMARY

When a tumor is found on a heart valve it may be either a Lambl excrescence, a product of an inflammatory reaction or a neoplasm. Organized thrombi do not have to be considered, since they do not occur on valves.

Lambl²⁷ first described the excrescences which bear his name as thread-like structures 2 to 3 mm. in diameter, occurring on the aortic valve. These are papillary in form and resemble myxomas microscopically. Koechlin²² felt that all the valvular myxomas should be called Lambl excrescences. In examining 150 bodies he found thread-like growths on the valves in 20 per cent of the cases. However, all the Lambl excrescences reported have occurred on the aortic cusps with the exception of 2 cases reported by Koechlin,²² in which they were found on the mitral valves, and in these there was evidence of an old endocarditis present. Whereas Lambl excrescences are generally found in a heart exhibiting endocardial changes myxomas occur in hearts with a normal endocardium. Moreover, myxomas are larger, measuring 6 to 10 mm. in diameter, and are more compact. Inflammatory growths on the valves are generally accompanied by gross thickening of the valves, destruction of valvular contours, and by microscopic scarring.

If it be assumed, as did Ribbert⁵ and Leonhardt,¹⁹ that true mucin occurs in a true tumor, and could come about in no other way, then these valvular growths can be readily differentiated by the various chemical and color reactions characteristic of mucin. Ribbert believed that the myxomas were true tumors which originated from embryonic connective tissue, and that ultimately the mucoid tissue gave rise to fibrous tissue. The fibroma described by Hagedorn⁸ represents the endpoint in this development.

Reitmann,¹⁸ on the contrary, believing that the myxomas represented a stage of degeneration of connective tissue tumors, designated his case as a hyalofibroma.

Whether the myxomas are really neoplasms, or whether they represent a degenerative process in connective tissue growths is still open to question. The fact is, however, that they do represent a group of tumors that occur in the heart, and have rather uniform morphological characteristics.

NOTE. I am greatly indebted to Dr. Francis Carter Wood and to Dr. Leila C. Knox for their encouragement and criticism throughout this work.

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DESCRIPTION OF PLATES

PLATE 104

FIG. 1. Heart showing a myxoma (A) attached to the tricuspid valve on the auricular surface. The right ventricle has been cut open. Approximately natural size.

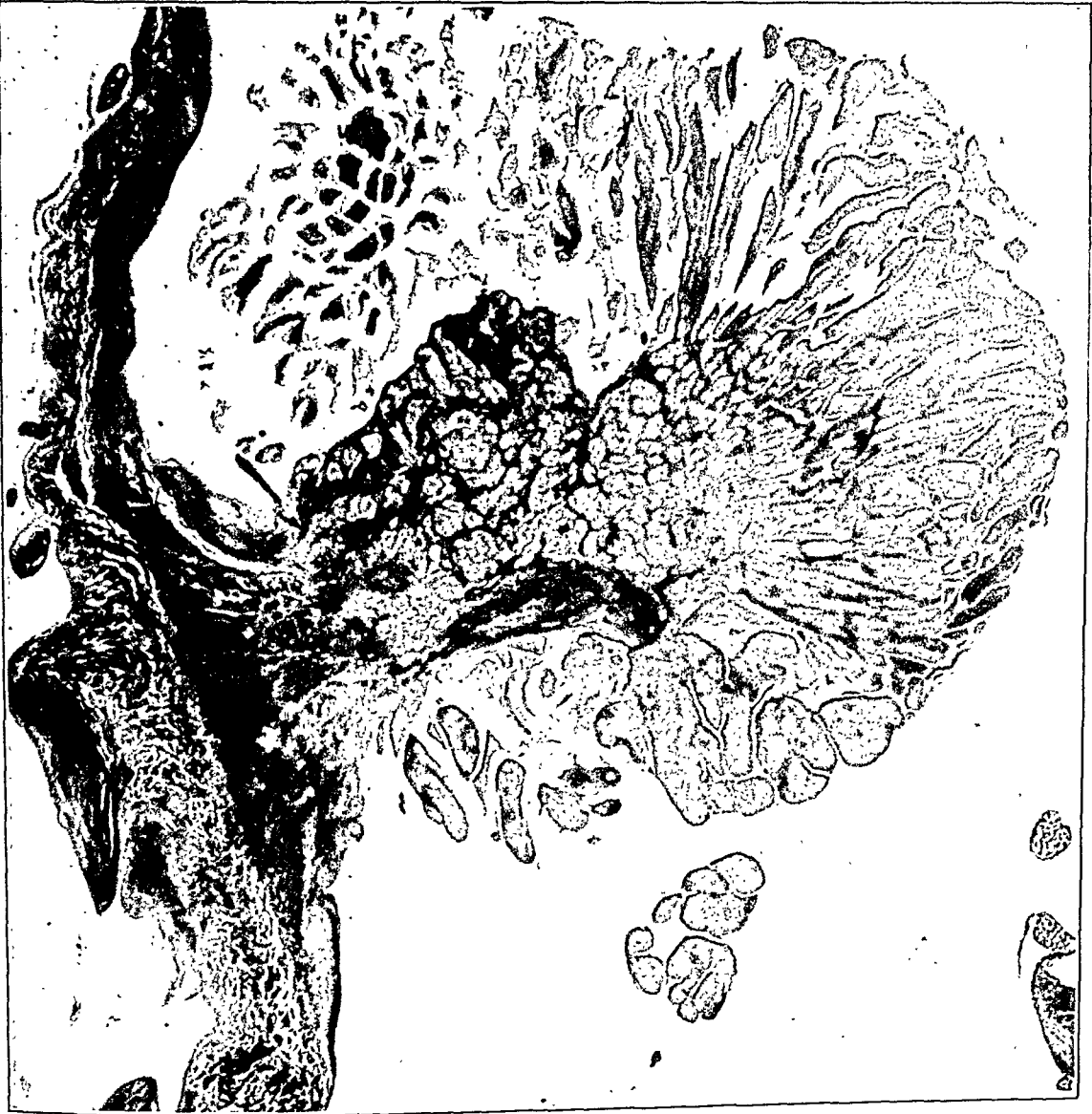


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PLATE 105

FIG. 2. Microscopic section showing a myxoma attached to the tricuspid valve.
× 18.

FIG. 3. Cross-section of several papillae of the myxoma showing the endothelial cells covering them, and the poorly cellular matrix. × 100.



2



3

MICROSCOPIC METASTASES IN THE THYROID GLAND *

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Metastases from malignant growths to the thyroid gland, although somewhat infrequent, probably occur more often than has been supposed. In a series of 521 autopsies on patients dying from carcinoma Müller¹ found gross metastases in the thyroid gland in 1.5 per cent. In another group of 102 postmortem examinations on patients dying from sarcoma he found metastases in 3.1 per cent of the thyroid glands. Kitain² found metastases in 3.1 per cent of the thyroid glands from autopsies on individuals dying of carcinoma. In 170 consecutive autopsies on persons with malignant tumors Willis³ found thyroid metastases in 5.2 per cent. He mentions that only by careful routine sectioning of the gland was it possible for him to find such a high incidence of metastases. In half of his 10 cases a casual bilateral section of the gland would have failed to disclose these metastatic areas. Wegelin⁴ does not consider metastasis to the thyroid gland an exceptional rarity, for he found it frequently in the material examined at the Pathological Institute of the University of Bern.

It is a well known fact that certain types of malignant conditions select certain regions for their metastatic growths. Carcinoma of the thyroid, kidney and prostate seems to have a selective affinity for metastasizing to bone, whereas carcinoma of the breast has a tendency to metastasize to the lungs.

The causative factor for this peculiar type of selective metastasis is not definitely established. Various mechanical theories have been suggested to account for the lodgement of malignant cells in certain areas. Likewise, to chemical factors has been attributed the principle rôle in determining the sites for the development of metastatic growths. Paget⁵ speaks of certain tissues as being "congenial soil," whereas Ewing⁶ has expressed an opinion opposed to this when he mentions that no organ is more adapted than any other for the growth of embolic tumor cells.

* Received for publication December 1, 1933.

Among the 57 autopsies with secondary carcinomatous growths in the thyroid gland reviewed by Willis, the primary tumor was most frequent in the breast. Next in frequency were the malignant melanomas, and third, carcinoma of the lungs and bronchi. He suggested that the high incidence of this phenomenon secondary to carcinoma of the breast was in all probability due to the greater frequency of that disease. On this assumption it should be possible to give first and second place to malignant melanoma and carcinoma of the lungs, both of which are relatively infrequent types of malignancy in the thyroid. Kaufmann⁷ and Eiselt⁸ found a relatively high incidence in malignant melanoma. In 34 autopsies with thyroid metastases Wegelin found 6 arising from carcinoma of the esophagus, 5 from carcinoma of the lung, 5 from melanosarcoma, 4 from carcinoma of the stomach, and the others from a variety of sources. He mentions that in the majority of instances the metastatic growths in the thyroid are visible as definitely circumscribed nodules.

Of all the men who have reported cases of malignant disease with metastases to the thyroid, none of them has found metastases that were not grossly visible. This paper is written for the purpose of demonstrating that microscopic metastases may occur in the thyroid gland without any macroscopic evidence of their presence.

Among 89 postmortem examinations from the department of pathology of the University of Minnesota on patients dying from malignant tumors there were embolic tumor cells within the thyroid gland in 9. The thyroid glands from all these patients were examined grossly and microscopically. Five of these showed gross metastases: 2 were from patients dying of malignant melanoma, 1 from carcinoma of the lung, 1 from lymphosarcoma, and 1 from carcinoma of the breast. These carcinomatous nodules were easily identified on macroscopic inspection of the gland. The remaining 4 cases presented, on gross inspection, no evidence of any tumor tissue or anything else to suggest the presence of malignant invasion. The essential details in the case histories of these cases are reported in the following case reports.

CASE REPORTS

CASE 1. Mrs. E. F., (A-29-595), aged 44 years, was admitted to the Minnesota General Hospital on March 28, 1929. A radical operation for carcinoma of the breast had been performed 3 months previously. The patient died on April 17, 1929.

The postmortem examination revealed numerous metastatic nodules in the bones, liver and lungs. The thyroid gland weighed 34 gm., and appeared normal in structure, with the exception of a few small colloid nodules and one large parenchymatous nodule measuring 15 mm. in diameter. There was no area, on gross inspection, that suggested malignant tissue.

Microscopic examination of the thyroid tissue reveals normal acini. Scattered throughout in the interlobular septa there are groups of large, irregularly shaped, deeply stained, closely packed cells, among which occasional mitotic figures can be seen. The nuclei are large and the cells contain a small amount of cytoplasm (Figs. 1 and 2).

The histological structure of the metastatic nodules from the liver is similar to the carcinomatous tissue observed in the interlobular septa of the thyroid.

CASE 2. Mr. F. R., (A-29-1323), case history not available.

The thyroid gland weighed 27 gm. It was diffusely homogeneous in structure and appeared to be entirely normal. Neither nodules nor areas suggesting a malignant condition could be identified on gross inspection.

Microscopic examination reveals normal thyroid acini. Here and there in the interlobular septa can be seen groups of large, pale staining, round and polyhedral cells. Some of these have large vacuolated nuclei and a large amount of cytoplasm. Mitotic figures are moderately abundant (Figs. 3 and 4).

CASE 3. Mrs. J. E., (A-29-285), aged 67 years, was admitted to the Ancker Hospital on Feb. 14, 1929, complaining of difficulty in breathing, dyspnea and a productive cough of 5 days duration. The most pronounced symptom was the cough. Dyspnea became more severe 2 days prior to admission to the hospital. She also gave a history of having had an ulcerated lesion on the right malar region for the past 9 months.

On physical examination a superficial, ulcerated lesion 1 cm. in diameter was found over the right malar region and a similar lesion over the right mandible. Examination of the chest revealed coarse moist râles over both lungs, but no evidence of consolidation. The blood pressure was 230/120. A clinical diagnosis of acute bronchitis, laryngitis, hypertension and carcinoma of the cheek was made.

The X-ray report of the chest disclosed infiltration and fibrosis in both lungs. This was suggestive of pneumoconiosis, associated with tuberculosis, and possibly bronchiectasis in both apices.

The patient became rapidly weaker and died on Feb. 16, 1929.

The postmortem examination disclosed the indurated, ulcerated lesion on the cheek, but no section was made of this to determine its exact nature. The liver was enlarged. Some old adhesions were found in the pleural cavity. There were 400 cc. of fluid in the left pleural cavity and 200 cc. of blood-tinged fluid in the right pleural cavity. A large focus of consolidation was present in the right lung, and several large, anthracotic and caseous lymph nodes were present at the hilum of both lungs. Microscopic examination of these tissues shows carcinoma. The lung was considered to be the site of the primary lesion. Its gross appearance did not suggest carcinoma and its presence was not suspected until sections were examined. The thyroid gland weighed 22 gm. Its structure was uniform. There were no nodules or any areas that could be interpreted as malignant on gross inspection.

The microscopic structure of the thyroid acini appears to be normal. Scattered throughout, in the interlobular septa, are found groups of large clear cells with enlarged granular nuclei and a pale staining cytoplasm. Mitotic figures are occasionally seen. Inter-cellular bridges are not observed. The cells are similar to those found in the sections from the lungs (Fig. 5).

CASE 4. Mrs. L. K., (A-30-754), aged 47 years, was admitted to the Ancker Hospital on April 25, 1930, complaining of distention with gas, loss of appetite and exhaustion for the past 6 weeks. She had had no pain, nausea, vomiting, or food distress.

Physical examination disclosed a slight bilateral exophthalmos. The liver was found to extend 7 cm. below the costal margin. A systolic murmur was heard along the sternum in the second left interspace. The knee jerks were hyperactive; Babinski test, normal.

On May 1, 1930, palpation revealed fullness in the left upper quadrant of the abdomen. The patient was drowsy and very restless. On May 4th she became stuporous and could not be aroused. She developed a left sided hemiplegia and became incontinent. The knee reflexes became increased on the left. The Babinski test was positive on the left. On May 8th there was definite neck rigidity. Pure blood was found in the spinal fluid. Two hard lumps appeared on the head, one of which was located over the left frontal bone, the other in the right parietal region. On May 12th many fine râles were heard in the lungs. The temperature varied from normal to 102.4 F. The pulse ranged from 80 to 140. The hemoglobin was 55 per cent, erythrocytes 3,290,000, lymphocytes 5600, differential count normal.

The patient became progressively worse and died on May 12, 1930.

At the postmortem examination it was found that the two nodules on the head were metastases in the cranium, extending into the

cranial cavity. Numerous grayish white nodules, which proved to be metastatic tumor tissue, were found within the substance of the liver, occupying approximately one-fifth of the liver volume. The gall-bladder contained numerous faceted stones and its wall was definitely thickened and almost cartilaginous. This tissue proved to be carcinomatous and was thought to be the primary lesion. There was an area of pneumonic consolidation in the lower lobe of the left lung. Numerous metastatic nodules were found around the otherwise normal pancreas. The preaortic and mesenteric lymph nodes were invaded with metastatic tumor tissue. Pus was found beneath the arachnoid over the right hemisphere and there was softening of the entire right half of the brain. The thyroid gland was homogeneous in structure and appeared grossly to be entirely normal. There was nothing to indicate the presence of metastatic tumor tissue.

Microscopically the thyroid acini appear normal. Scattered throughout in the interlobular septa can be seen groups of large basophilic cells with large, round and polyhedral nuclei, and a moderate amount of pale staining cytoplasm. In other places these cells are darker and more spindle-shaped. Mitotic figures are abundant. The malignant cells are similar to those found in the gall-bladder, liver and lymph nodes (Fig. 6).

DISCUSSION AND CONCLUSIONS

These cases illustrate the occurrence of a type of metastasis that is often thought of as a precursor to the gross metastatic nodules, but which is infrequently seen in pathological specimens, probably because of the prevailing tendency to make histological sections only where gross pathological changes are visible.

In all these cases macroscopic inspection of the gross specimen failed to give any intimation that there were metastases in the thyroid gland. Although it is probable that this type of metastasis occurs frequently, a search of the literature has failed to reveal any reports of a similar nature.

The incidence of gross metastases in the thyroid gland, as reported by other writers, ranges from 1.5 per cent to 5 per cent. These figures compare favorably with those in this report when the cases with microscopic metastases are not included, but otherwise

the incidence approaches 10 per cent. It may be assumed then that microscopic metastases in the thyroid gland occur almost as frequently as gross metastases in this organ, and in all probability the incidence of metastasis is much greater than has been reported in the literature.

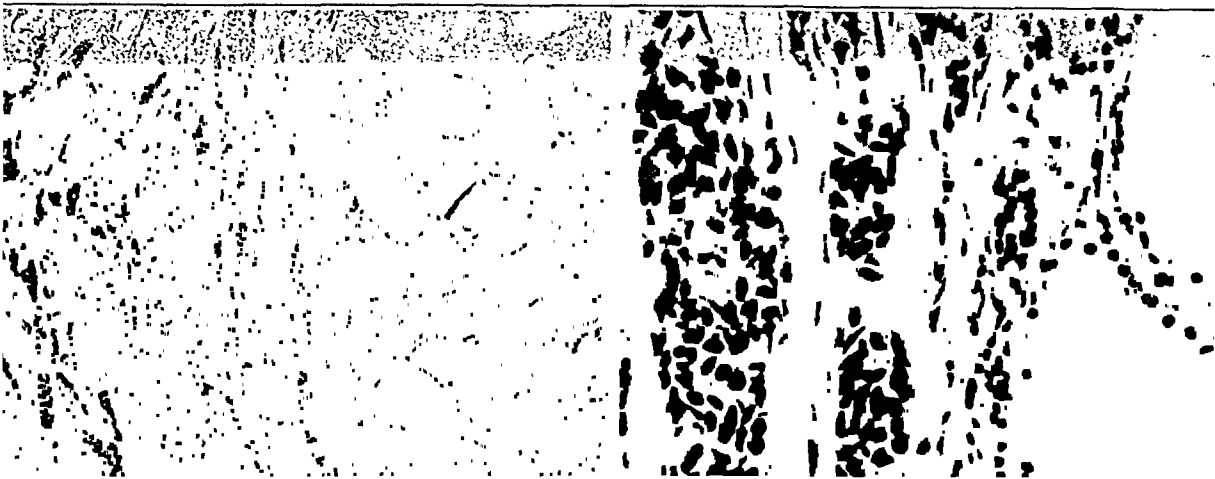
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DESCRIPTION OF PLATE

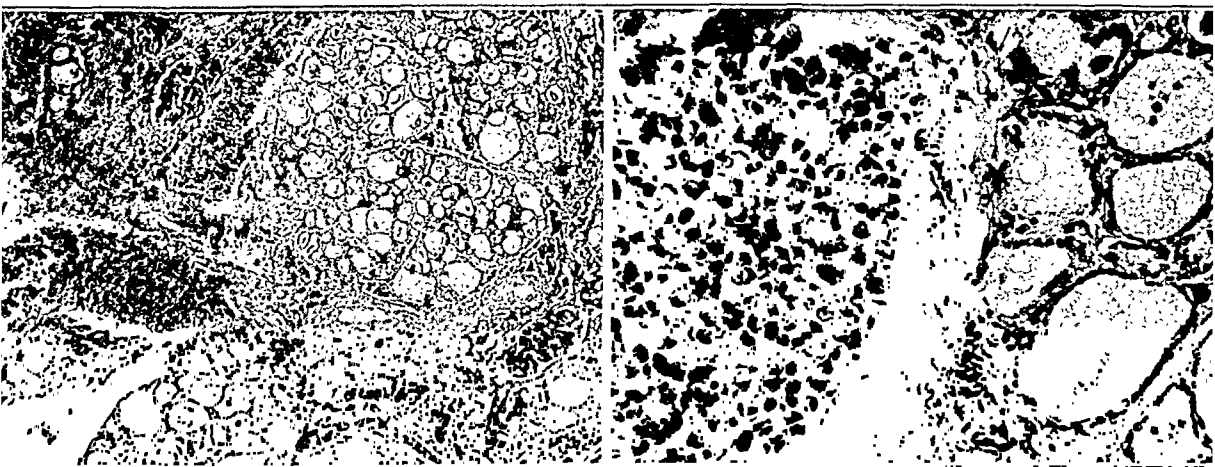
PLATE 106

- FIG. 1. Case 1. Photomicrograph showing the microscopic structure of the thyroid gland with groups of deeply stained carcinomatous cells invading the interlobular septa.
- FIG. 2. Case 1. High power magnification from the same section as Fig. 1, depicting a few of the groups of tumor cells. Two thyroid acini are seen at the right of the picture.
- FIG. 3. Case 2. Photomicrograph showing interlobular septa filled with large and small groups of tumor cells. Thyroid acini normal.
- FIG. 4. Case 2. High power magnification from the same section as Fig. 3. Mitotic figures are seen on the left side.
- FIG. 5. Case 3. Photomicrograph showing thyroid acini which appear normal. The interlobular septa is occupied by groups of carcinomatous cells.
- FIG. 6. Case 4. Photomicrograph showing interlobular septa filled with densely packed groups of tumor cells. Thyroid acini appear normal.



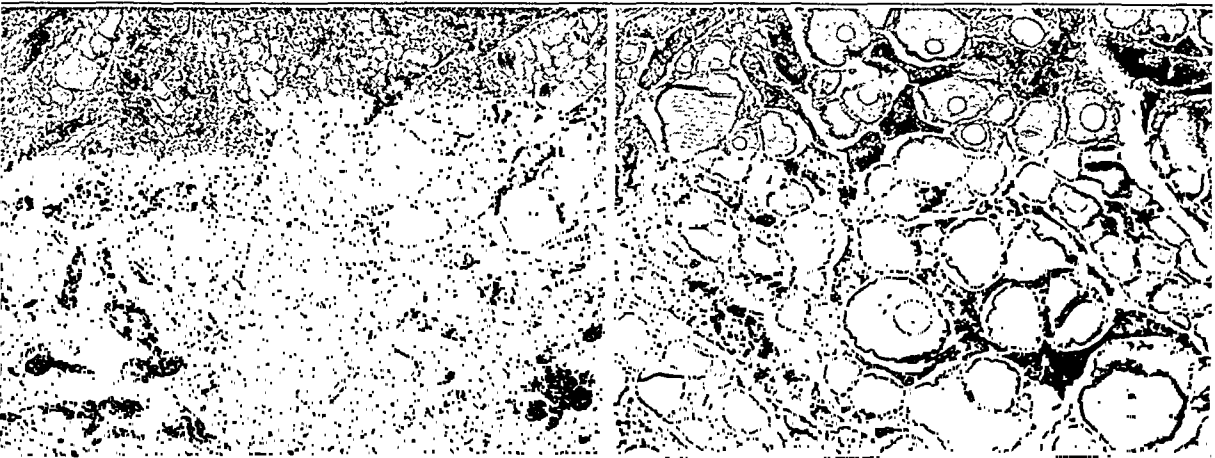
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SARCOSPORIDIA IN THE MYOCARDIUM OF A PREMATURE INFANT *

REPORT OF A CASE

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REVIEW OF LITERATURE

Scott,¹ in a critical review of the sarcosporidia, summarizes our present knowledge of this group of parasites. The sarcosporidia are protozoa of unclassified position, found almost exclusively in the striated muscles of birds, reptiles and mammals, including man. Miescher² in 1843 first described the parasite in the muscles of the mouse. The genus *Sarcocystis* was created in 1882 by Lankester.³ Since then at least 43 species have been described from an almost equal number of hosts. Whether these are all true species or whether only one or a limited number of species exists is not known. Probably there are several species, since some are transmitted to other hosts with difficulty. Darling⁴ and others, however, have shown that at least two hosts may be parasitized by the same species, although in the transfer to a new host certain morphological changes occur in the parasite.

In the opinion of most investigators natural infection is through the digestive tract by means of contaminated food, although the exact details are not known. The first successful transmission by feeding was obtained by Theobald Smith⁵ in 1901, who was able to infect 63.6 per cent of gray and white mice by feeding them muscle from previously infected animals (mice). Nègre⁶ and Crawley⁷ have both shown that feces of infected mice are capable of causing the disease when ingested by other mice. The infective stage in this instance is a spore, resistant to heat and drying. Erdmann⁸ stated that in the intestinal tract the spore liberates a small ameboid form which penetrates the epithelium. It then multiplies within the various portions of the gut wall and surrounding lymphatics to ap-

* Received for publication December 6, 1933.

pear finally 28 to 30 days later in the striated muscles. The various stages within the striated muscle fibers are known largely from the works of Theobald Smith,⁵ Scott¹ and others.

HUMAN CASES

The first probable instance of infection with sarcosporidia in man was reported by Lindemann⁹ in 1868, as occurring in the heart valves and myocardium. These were interpreted as gregarines but Fantham, Stevens and Theobald¹⁰ consider them sarcosporidia. The latter authors doubt the authenticity of Rosenberg's case of sarcosporidia,¹¹ occurring in the mitral papillary muscles in the heart of a woman 40 years of age, dying with pleuritis and endocarditis. Fantham, Stevens and Theobald also cite 2 cases reported by Koch in 1887, and by Vuillemin in 1894, respectively. The location of the parasite in the case reported by Koch was not stated, while in the case reported by Vuillemin the parasite occurred in the voluntary muscles of a patient dying of tuberculosis. Cone's report¹² in 1922 of the parasites associated with yeasts in multiple bone cysts is a disputed case. The undoubted cases occurring in the human are best summarized in the accompanying table.

It is the purpose of this paper to report the incidental finding of a sarcosporidial infection in the myocardium of a premature infant.

REPORT OF CASE

Clinical History: A 10 day old, white, female infant, which was born 2 months prematurely, was brought to the hospital with a history of having had loose stools since birth and ulcerations of the buttocks for 2 days. Since birth it had been fed by a medicine dropper on a very weak, whole milk, dextri-maltose and water formula. Physical examination revealed emaciation, and ulceration of the buttocks. On the 6th day after admission an omphalitis became apparent, with discharge of thick yellow purulent material from the umbilicus. The latter, along with the diarrhea, became progressively worse and the baby died 16 days after admission with signs of a terminal bronchopneumonia and cardiac dilatation.

POSTMORTEM EXAMINATION

Postmortem examination (A-32-163) revealed a poorly nourished premature infant measuring 40 cm. in length. Purulent exudate filled the proximal end of the umbilical vein. Multiple abscesses yielding *Staphylococcus aureus* and *Bacillus coli* occurred in the cortex

of both cerebral hemispheres, and to a less extent throughout the brain. The lungs showed a bilateral terminal bronchopneumonia with atelectasis and compensatory emphysema. Microscopic foci of necrosis were found in the myocardium, liver and spinal cord. Small

TABLE I
*Undoubted Cases of Sarcosporidiosis **

Author	Year	Age	Organ
Kartulis ¹³	1893	yrs. Adult	Abdominal muscles
Baraban and Saint-Remy ¹⁴	1894	Adult	Laryngeal muscles
Darling ¹⁵	1909	20	Biceps
Darling ¹⁶	1920	30	Tongue
Manifold ¹⁷	1924	Adult	Myocardium
Lambert ¹⁸	1927	32	Myocardium
** Vasudevan ¹⁹	1927	Adult	Pectoral muscles
** Naidu ²⁰	1928	55	Pectoral muscles
Bonne and Soewandi ²¹	1929	Adult	Cavernous hemangioma
Hewitt ²²	1933	Adult	Myocardium

* An additional human case of sarcosporidiosis, reported by Price, R. M., in the *J. Kansas M. Soc.*, 34, 132-135, has come to my attention since this paper was written.

** These two possibly represent reports of the same case.

TABLE II
Comparative Measurements of Parasite in μ made by Various Authors

Author	Cysts		Spores	
	Length	Diameter	Length	Diameter
Baraban and Saint-Remy ¹⁴	150-1600	77-168
Darling (Case 1) ¹⁵	84	27	4.0	1.0
Manifold ¹⁷	37-57	26-45	10.9	1.6
Lambert ¹⁸	82	31	7.2	2-2.5
Vasudevan ¹⁹	5.3 cm.	322	3.3	1.6
Naidu ²⁰	195-240	...	12.7	4.4
Hewitt ²²	190	50	5.0	2.0
Hertig.....	11.2-45	7.4-13	3.7	1.8

foci of purulent meningitis were found over the cerebral cortex, as well as a slight acute inflammatory reaction throughout the brain substance. The lungs, in addition to bronchopneumonia, gave microscopic evidence of aspirated amniotic sac contents. The pancreas showed slight, diffuse, acute inflammation with inspissation

of secretion in the finer ducts. Moderate numbers of sarcosporidia, including many in the early stages, were found microscopically in the myocardium. In no instance was there cellular infiltration in response to the presence of the parasite, although independent foci of necrosis were found. Examination of other striated muscle (diaphragm) revealed no parasites.

DESCRIPTION OF PARASITE

The lateral portion of the left ventricle, taken during a routine postmortem examination, was the source of the material. The parasites were identified by Professor S. B. Wolbach in studying the microscopic preparations. Sections stained by eosin and methylene blue, from material fixed in Zenker's fluid, gave the clearest histological detail, although hematoxylin and eosin, Giemsa, iron hematoxylin and Foot's reticulum stains were also made. The parasites were moderately numerous throughout the myocardium, averaging 1 per low power field. They lay within the myocardial fibers as sharply demarcated, oval bodies varying from 7.4 by 11.2μ to 13 by 45μ , with the long axis parallel to that of the surrounding fiber. The parasites, as seen in individual sections, were composed of closely packed, oval or spindle-shaped spores varying from 6 to 100 in number. No definite wall could be seen in the forms possessing less than 13 spores, although beyond that stage a hyaline wall or capsule averaging less than 1μ in thickness was present. Occasionally, due to artifact, the cyst wall was pulled away from the surrounding muscle fiber and thus could readily be seen. No septa were seen in any of the forms studied. Slightly over 30 per cent of the parasites in this preparation contained from 6 to 13 spores, with the higher numbers predominating slightly, although approximately 10 per cent contained between 50 and 100 spores. The spores averaged 1.8 by 3.7μ in size. The basophilic nuclear mass was irregular and occupied an eccentric position, often filling one end of the spore. No nuclear membrane could be made out, although the spore membrane was quite definite. Very rarely a suggestion of a minute extranuclear basophilic mass could be seen at the end of the spore opposite the nucleus. At no place in the myocardium was there any inflammatory response to the parasites. The remainder of the myocardium was essentially negative, except for foci of necrosis associated with the staphylococcus septicemia.

DISCUSSION

This case is of interest because of its occurrence in a premature infant and the early stage of development of the sarcosporidial cysts. Even in experimental studies it is uncommon to find sarcocysts with fewer than 8 spores, although moderate numbers of the forms studied here were of this type. The method of infection is unknown. However, since the infant was 26 days of age at death the infection could have been contracted shortly after birth, because the stage of development coincides very well with that seen in animals 26 to 29 days after ingestion of the infective spores. Theobald Smith²³ and Scott¹ state that intra-uterine infections do not occur in the lower animals, although this method cannot be ruled out in this case. Since mouse feces are known to be infective and since indigenous mouse infections may be common, this might have been a source of infection.

SUMMARY

A case of sarcosporidiosis involving the myocardium of a 26 day old premature infant is reported. This was an incidental finding in the routine microscopic study of postmortem material. The mode of entrance of the parasites into the body cannot be stated with certainty.

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DESCRIPTION OF PLATE

PLATE 107

FIG. 1. Sarcosporidial cyst in the myocardium of a premature infant. $\times 1300$.



I

OCCURRENCE OF AMYLOIDOSIS IN RABBITS EXPERIMENTALLY INFECTED WITH TUBERCULOSIS *

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Amyloid degeneration of the spleen, liver and kidneys has long been known to occur in the course of chronic wasting diseases, particularly in those in which suppuration is present. Chronic tuberculosis fulfills both of these conditions, and most cases of amyloid degeneration are found in this disease. Higuchi,¹ in assembling the autopsy findings of his own and of other European hospitals, found in a total of 72,050 autopsies 1169 cases of amyloid degeneration, of which 75.5 per cent were in tuberculous cases. Von Schrötter,² in a series of 3716 tuberculous autopsies, found 221, or approximately 6 per cent, affected with amyloid degeneration. In contrast to this are the figures published by Cummins³ from a smaller series, in which he found 123 cases of amyloid degeneration in a total of 236 autopsies on tuberculous patients. It may be noted that Cummins stated in his publication that he used a specific stain (methyl violet) in examining the sections, whereas the above authors make no note of having used specific stains. Excellent reviews of the chemistry and of the histopathology, together with bibliographies of the older literature, have been published by Schmidt,⁴ Wells and Long,⁵ Edens^{6,7} and Davidsohn.^{8,9}

The material for the present study consisted of a group of 175 rabbits that had been infected with bovine tubercle bacilli and allowed to die of their infection. Gross and microscopic examination of the tissues was made in each case. Sections were stained with hematoxylin and eosin as routine, and in addition with azocarmine and aniline blue (Heidenhain's modification of Mallory's aniline blue stain) and, in a few instances, with Congo red according to the method of Bennhold.¹⁰ The average length of life of the animals after infection was 6.7 months, with a lower limit of 2 weeks and an upper limit of 20 months (see Chart 1).

* Received for publication December 7, 1933.

Rabbits that died during the first 2 months after infection did not show any amyloid changes, despite the fact that they were suffering from a widespread tuberculosis. It may be noted in this connection that at this time caseation is not a pronounced factor. In the 3rd month the incidence of amyloidosis was 23 per cent, in the 4th month 55 per cent, and in the succeeding months between 60 per cent and 87 per cent. Of all the animals that survived beyond 2 months after infection 62 per cent showed amyloid degeneration of one or more organs, while of those that survived 8 months or

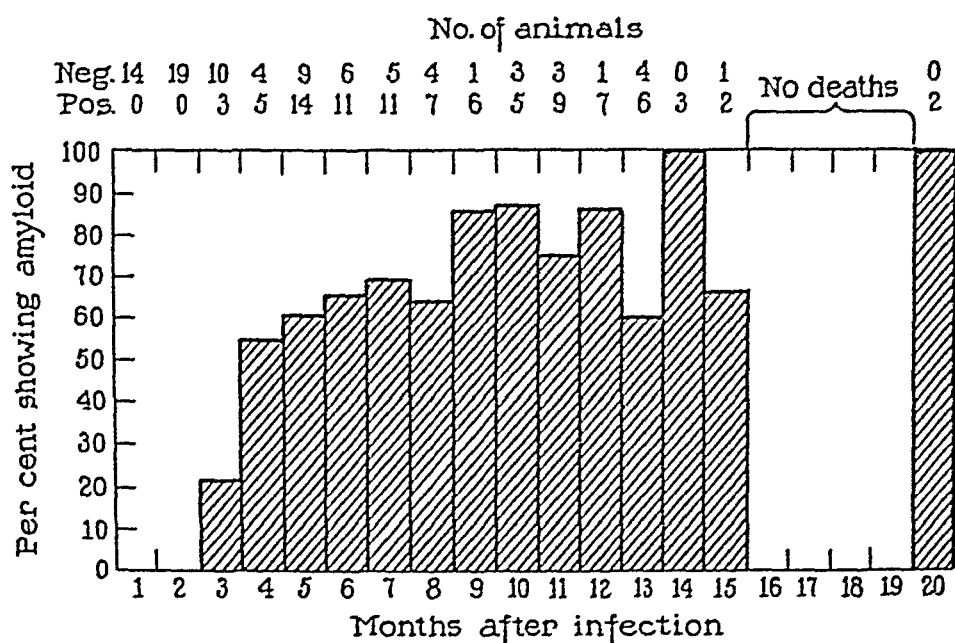


CHART 1

longer 75 per cent were involved. The incidence by months is graphically shown in Chart 1.

The spleen, liver, and kidneys showed the greatest involvement. The mesenteric lymph nodes were never extensively involved and in but a few cases was any amyloid found in the lymph nodes. The spleen was most frequently involved, and in a number of animals was the only organ affected. The early changes in the spleen consisted of a deposition of amyloid substance in the capillary walls at the edge or border of the malpighian corpuscles, forming in section a "rim" around the follicle. Later, extension occurred toward the center of the follicle, leaving finally the central artery with but a remnant of lymphoid tissue about it. The capillaries of the follicle, surrounded as they were by masses of amyloid substance, fre-

quently remained open and did not show constriction of their lumens. In approximately half of the cases the amyloid was not confined to the follicles but had also involved the pulp, so that the entire organ was a mass of amyloid material. There was no increase in weight of the spleens in the affected animals. The average spleen weight in the affected group was 1.5 gm., and in the unaffected group 1.56 gm.

In the liver amyloid deposits were found between the endothelium lining the liver sinusoids and the liver cells, and occasionally in the media of the central veins. In severe cases the liver cells were thinned out and practically obliterated by the encroachment of the amyloid substance upon them. No evidence of vascular obstruction was noted in any of the cases, although this feature, terminating in extensive hemorrhage and rupture of the liver, is common in "anti-toxin" horses that develop amyloid disease of the liver.^{11, 12}

The kidneys were found to be less frequently involved than the spleen or liver. Amyloid deposits were found in the capillary tufts of the glomeruli, sometimes involving but one loop and sometimes the entire glomerulus. In several severe cases all of the glomeruli were so involved, with attendant atrophy and degeneration of the tubules.

The kidneys and livers of these animals were not weighed at autopsy. There were no gross evidences of amyloidosis save for an increased firmness of the organ. Microscopically there was no evidence that in either the liver or kidney amyloid deposits tended to form in large aggregates.

The clinical course of the disease seemed not to be affected by the occurrence of amyloidosis. The changes in the white blood cells and the red blood cell count were not significantly different in the two groups. There was no increased cachexia in the affected group of animals. As regards the effect of amyloid degeneration of the spleen, liver and kidneys upon the length of survival of the animals after infection, it is seen in Chart 1 that the distribution of amyloidosis was not confined to any age group; and that the unaffected group (exclusive of those that died in the first 2 months) lived an average of 6.7 months, while the group in which amyloid was found survived an average of 8.5 months. These figures indicate that amyloid changes are a function of chronicity rather than an untoward incident or complication in the course of the infection. On the other hand, the examination of the autopsy records showed that

amyloid changes were more frequently found in those animals with extensive caseation. While the extent of pulmonary tuberculosis was approximately the same in the two groups, in the unaffected group only 23 per cent showed tuberculosis with caseation of the testicle and 64 per cent similar tuberculosis in the kidney. In the animals with amyloid changes, however, the testicles were involved in 96 per cent of the cases and the kidneys in 81 per cent. In rabbits experimentally infected with tuberculosis the testicles and kidneys are both prone to undergo rapid and extensive caseation and in severe cases may together contain 50 to 60 gm. of caseous material.

In but 3 cases did there appear to be sufficient involvement of the liver or kidney to account for the death of the animal. The fact that the group of animals that showed amyloid degeneration and more extensive lesions with caseation survived, as a group, longer (1.8 months) than the unaffected group is but an expression of the fact that amyloid degeneration is a function of chronicity, so that given indefinite survival the incidence would approach 100 per cent. The relation between extent of disease and survival time after infection with tuberculosis is by no means clear, and this is particularly true in experimental tuberculosis where the actual causes of death are frequently obscure.

SUMMARY

1. Amyloid degeneration occurred in 52 per cent of 175 rabbits experimentally infected with bovine tubercle bacilli.
2. The occurrence of amyloidosis was restricted to animals surviving longer than 2 months after infection.
3. The frequency of occurrence was greatest after the 8th month (75 per cent).
4. The organs affected were the spleen, liver and kidney, the spleen being most frequently affected.
5. There was a uniform tendency for the deposition of amyloid to occur in those animals that showed the most extensive caseation of their lesions.

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A STUDY OF THE ACTION OF A FILTRABLE STAPHYLOCOCCAL TOXIN ON THE KIDNEYS OF NORMAL RABBITS *

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During a study of the action of a staphylococcal toxin on leukocytes *in vivo* and the subsequent changes produced in the femur bone marrow of normal rabbits¹ it was noted at the time of autopsy that some of the animals had extensive necroses of the cortical portion of the kidneys. It was thought that this observation deserved further study and this is the experiment to be submitted.

So far as we have been able to determine Neisser and Levaditi² in 1900 were the first to produce necrosis in the kidneys of rabbits by the intravenous injection of staphylococcal toxins. Following the publication of this work little attention was given to staphylococcal toxins until the revival of interest, which began a few years ago following the experiments of Julia T. Parker Weld. Subsequent to her reports we have been able to find only two references to necrosis in kidneys after the intravenous injections of staphylococcal toxins: one was by Weld and Gunther³ in 1931, and the other was by Forssmann⁴ in 1932. From a study of the literature it is evident that the kidney lesions produced by the intravenous injections of staphylococcal toxins have not received due consideration.

METHODS AND MATERIALS

Animals: The animals used in these experiments were normal adult rabbits.

Toxin: The toxin was prepared by the method described by Parker, Hopkins, and Gunther,⁵ with a few unessential modifications.

Organism: A hemolytic *Staphylococcus aureus* was the organism from which the toxin was prepared. This staphylococcus had been isolated from the throat of a patient who presented the classical lesions found in agranulocytic angina. The cultures of this organism were grown on blood agar.

Procedure: The rabbits were given either a single intravenous injection of toxin each day until death, or the injections were made daily over a period of 10 to 12 days. The initial dose was usually 0.5 cc. with subsequent increase in dosage to as high as 5 cc.

* Received for publication December 11, 1933.

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The usual procedure was to inject from 0.5 to 3 cc. of the toxin intravenously and study the kidneys at autopsy. In a few cases one kidney was removed 6 days after a single injection of the toxin and the animal was killed 13 days later for comparison of the kidney lesions.

Control Experiment: Sterile peptone broth was injected intravenously into the ear veins of normal rabbits for control of our experiment. The time of the injections and the amount of broth corresponded to the injections of the toxin.

Blood Analysis: Chemical analyses were made on the blood of rabbits to determine the carbon dioxide combining power of the blood and the content of non-protein nitrogen, chlorides, glucose and uric acid. Two cc. of blood were usually taken before each injection of the toxin. It was impossible to obtain a sufficient quantity of blood each day for the above determinations. Ten to 15 cc. of blood were obtained from the ear veins 4 to 5 days before the toxin was given and a similar amount was taken from the heart a few minutes before death. Since non-protein nitrogen of the blood was the only element that showed any constant variation from the normal, the remaining constituents that were determined are not included in this report.

Autopsies: A complete autopsy was performed and the tissues were fixed in Zenker-formalin and formalin.

Stains: Sections of the kidneys were stained routinely with hematoxylin and eosin and certain sections were stained for connective tissue by Mallory's aniline blue method and with scharlach R for fat.

EXPERIMENTAL

Eighty rabbits were given injections of staphylococcal toxin and of this group 58 animals died, and the other 22 rabbits were killed. Eighteen rabbits received intravenous injections of sterile peptone broth without a single death. The amount of toxin given and the length of time elapsing before death varied in the groups of rabbits. Some of the animals died immediately after the intravenous injection of 0.5 cc. of the toxin, while other rabbits received as much as 22.5 cc. over a period of 9 days before death occurred. The animals that received an initial dose of 2 to 3 cc. of the toxin usually died in 3 to 5 hours. Three rabbits that were given 0.5 cc. of the toxin and operated upon 6 days later showed only microscopic lesions in the kidneys, which will be described below.

The acute lesions produced in the kidneys varied in extent; however, the general process was always the same. The lesions may be divided into three groups.

In Group 1 are those rabbits that died within 12 hours after the first injection of the toxin. The kidneys of these animals were normal in the gross. In the microscopic sections the glomerular tufts are swollen and the capillary loops are dilated and filled with red

blood cells (Figs. 1 and 2). In some of the glomeruli there is an accumulation of an albuminous material in the space between the tuft and the capsule. The epithelial cells lining the tubules are swollen, especially in the convoluted tubules and in the loop of Henle. These swollen cells not only diminish the size of the lumens of the tubules but often occlude them. In the lumens of some of the tubules there is some albumin.

In Group 2 the kidneys in the gross were swollen and purplish red in color; on the cortical surface small, irregular, yellow areas, which extended downward into the deeper layer of the cortex, could be seen. In the microscopic sections the epithelial cells lining the tubules are swollen and necrotic and often form epithelial casts. Many of the epithelial cells that remain are filled with large hyaline droplets. In addition to the desquamated epithelial cells in the tubules there are many hyaline casts, hyaline droplets and albumin. A few red blood cells are present in the lumens of some of the tubules. The endothelial cells are slightly swollen in the capillary loops of the tufts; however, the loops are not distended with red blood cells, but show a few hyaline thrombi. In the space between the tuft and the capsule in some glomeruli there are occasional large degenerated cells and a few red blood cells. Polymorphonuclear leukocytes and large mononuclear cells are present in some of the glomeruli and similar inflammatory cells are present in areas of the interstitial tissue. The yellow areas noted in the gross (Fig. 5) in the cortex of the kidneys have the appearance of infarcts, although only rarely are blood vessels occluded by thrombi. The glomeruli, tubules and interstitial tissues are necrotic and many polymorphonuclear leukocytes are present in some of the yellow zones (Fig. 6).

In Group 3 the kidneys resembled grossly those in the second group; however, the cortical surface here was yellowish brown in color and the necrosis was diffuse, involving the entire cortex. When the kidneys were sectioned there was a definite line of demarcation between the necrotic cortex and the better preserved medulla. The histological changes in the kidneys in this group are essentially the same as described in Group 2; however, the process is more diffuse.

In the group that received a single injection of 0.5 cc. the kidneys removed 6 days later were grossly normal; however, microscopically they show focal areas in which the epithelial cells lining the tubules have undergone degeneration (Fig. 3). That these lesions are of a

transient nature is indicated by the fact that examination of the remaining kidney, removed at autopsy 13 days later, shows no abnormalities (Fig. 4).

The kidneys in Rabbit 1, which was killed on the 11th day after the first injection, were moderately swollen and the cortex was yellowish gray in color. Microscopically the tubules are filled with hyaline casts and desquamated tubular epithelial cells (Figs. 7 and 8). The non-protein nitrogen was 120 mg. per cent at the time of death. Many epithelial cells are absent along the wall of the tubules but the remaining epithelial cells are not swollen, as was noted in the kidneys in those rabbits dying in the acute stage.

Rabbit 3 was killed on the 19th day after the first injection of the toxin. The kidneys in the gross had small hemorrhagic areas and small gray areas on the cortical surface. Histologically a few of the glomeruli are found to have blood in the space between the tuft and the capsule.

A chemical analysis of the blood was made on a series of animals that received the staphylococcal toxin (Table I) and also upon the control group that received only sterile peptone broth (Table II), in order to determine whether there was any relation between nitrogen retention in the blood and the extent of damage in the kidneys. The majority of the rabbits receiving injections of the toxin showed a gradual elevation of the non-protein nitrogen until death, whereas the control group showed neither nitrogen retention nor histological evidence of renal damage.

The rabbits that lived for a period of time after the intravenous injection of toxin grew progressively weaker during the last few hours of life and some of the animals had convulsions during this period. The rabbits presented the same clinical symptoms as those described by Weld and Gunther³ and Burnet.⁶ A majority of the rabbits that showed necrosis of the kidneys had a decrease or a complete absence of urine in the bladder at the time of autopsy. From this observation it would seem that there occurred a partial or complete suppression of urine.

The amount of toxin necessary to produce death and the length of time elapsing before death were variable factors. Because of this variation a standard dose of toxin could not be determined. Frequently death occurred within the first 24 hours after the intravenous injection of only 0.5 cc. of toxin.

We were unable to produce chronic renal lesions, for the animals that received a sufficient quantity of toxin to produce any degree of necrosis in the kidney died within a few days.

TABLE I

Summary of the Results in 4 Rabbits that are Selected to Illustrate the Changes in the Non-Protein Nitrogen of the Blood After the Intravenous Injection of the Staphylococcal Toxin (Normal Non-Protein Nitrogen 30-45 Mg.).

Rabbit 1				Rabbit 3			
Day	Time	Toxin	Non-protein nitrogen	Day	Time	Toxin	Non-protein nitrogen
		cc.	mg. per cent			cc.	mg. per cent
1	3 P.M.	..	44	1	4 P.M.	..	43.4
4	2 P.M.	0.5	..	2	2 P.M.	0.5	..
5	2 P.M.	..	49	3	9 A.M.	..	60
5	2.30 P.M.	0.5	..	3	11.30 A.M.	0.5	..
6	2 P.M.	1	..	4	10.30 A.M.	..	71
7	10 A.M.	..	48	4	12 NOON	0.5	..
7	11 A.M.	1.5	..	5	9 A.M.	..	91
8	10 A.M.	2	..	7	9 A.M.	..	48.4
9	9 A.M.	3	..	18	1.30 P.M.	..	44.3
10	9 A.M.	4	..	19	9.30 A.M.	..	39.8
11	9.30 A.M.	5	..	20	9.30 A.M.	..	38.4
12	11 A.M.	5	..	21	8.30 A.M.	..	38
14	2.30 P.M.	..	120	21	1.30 P.M.	..	37.5
14	2.30 P.M.	Killed	..	21	1.30 P.M.	Killed	..
Both kidneys are moderately swollen and the cortex is yellowish gray in color. The tubules are filled with hyaline casts and desquamated epithelial cells.				The cortex of the kidneys shows small hemorrhagic areas and depressed gray areas. A few red blood cells are present in some of the glomerular spaces.			
Rabbit 2				Rabbit 4			
Day	Time	Toxin	Non-protein nitrogen	Day	Time	Toxin	Non-protein nitrogen
		cc.	mg. per cent			cc.	mg. per cent
1	11 A.M.	..	36.3	1	4 P.M.	..	39.7
4	2 P.M.	0.5	..	2	2 P.M.	0.5	..
5	9.30 A.M.	1	..	3	9 A.M.	..	46.4
5	5 P.M.	..	80.8	3	11.30 A.M.	1	..
6	8.30 A.M.	2	..	4	10.30 A.M.	..	96
6	9.30 A.M.	..	75.4	5	11 A.M.	..	112
6	9.30 A.M.	..	Died	5	11 A.M.	..	Died

Both kidneys show cortical necroses.

Both kidneys show cortical necroses.

TABLE II

Summary of the Results in 3 Rabbits that are Selected from the Group Receiving the Sterile Peptone Broth. The Non-Protein Nitrogen of the Blood is always Within Normal Limits

Rabbit 5				Rabbit 6				Rabbit 7			
Day	Time	Broth	Non-protein nitrogen	Day	Time	Broth	Non-protein nitrogen	Day	Time	Broth	Non-protein nitrogen
1	4 P.M.	cc.	mg. per cent	1	4 P.M.	cc.	mg. per cent	1	4 P.M.	cc.	mg. per cent
4	12 NOON	1	39.5	4	12 NOON	1	31.2	4	12 NOON	1	48
5	10.30 A.M.	5	10.30 A.M.	5	10.30 A.M.
5	12 NOON	1.5	39.6	5	12 NOON	1.5	40.2	5	12 NOON	1.5	42.6
6	10.30 A.M.	6	10.30 A.M.	6	10.30 A.M.
6	11.30 A.M.	2	44	6	11.30 A.M.	2	39.6	6	11.30 A.M.	2	36.4
7	8.30 A.M.	7	8.30 A.M.	7	8.30 A.M.
7	9.30 A.M.	3	42.5	7	9.30 A.M.	3	36.7	7	9.30 A.M.	3	36.2
8	8.30 A.M.	8	8.30 A.M.	8	8.30 A.M.
8	10 A.M.	4	40.5	8	10 A.M.	4	35	8	8.30 A.M.	..	38.7
9	2 P.M.	9	2 P.M.	9	10 A.M.	4	..
9	2.30 P.M.	5	39.8	9	2.30 P.M.	5	34.5	9	2 P.M.	..	33.4
10	8.30 A.M.	10	2.30 P.M.	10	2.30 P.M.	5	..
13	8.30 A.M.	..	40	10	8.30 A.M.	..	35.8	10	8.30 A.M.	..	35.2
14	8.30 A.M.	..	39.5	16	1.30 P.M.	..	37	13	8.30 A.M.	..	38
16	8.30 A.M.	..	41.3	17	10 A.M.	..	38.8	14	8.30 A.M.	..	40.9
17	1.30 P.M.	..	38.2	17	10 A.M.	Killed	..	16	1.30 P.M.	..	41
17	10 A.M.	..	40	The kidneys are normal both grossly and microscopically				17	10 A.M.	..	35.7
17	10 A.M.	Killed	..	The kidneys are normal both grossly and microscopically				17	10 A.M.	Killed	..
The kidneys are normal both grossly and microscopically				The kidneys are normal both grossly and microscopically				The kidneys are normal both grossly and microscopically			

DISCUSSION

Neisser and Levaditi² described in detail the character of the necrosis occurring in the rabbit's kidney after the intravenous injection of staphylococcal toxin, and Neisser and Wechsberg⁷ state: "These changes can only be interpreted as infarcts which are sufficiently explained by the finding of thrombosed vessels, which as the microscopic picture shows, are caused by the rich disintegration of leukocytes which in turn we must look upon as a consequence of the leukocidin effect."

Although there is a destruction of leukocytes in the circulating blood¹ we have been unable to demonstrate thrombi composed only of leukocytes and, furthermore, it is our opinion that the process of infarction will not account for all the changes produced in the kidney after the intravenous injection of the staphylococcal toxin.

It has been suggested that histamine is present in the staphylococcal toxin in a sufficient quantity to produce the necrosis in the kidney. Dolman⁸ states: "Histamine has been shown to be present in the toxin in amount strictly comparable to that present in the original nutrient broth. Such amounts of histamine could not possibly be responsible for any of the characteristic features of the toxin. Moreover, if the histamine content of the toxin be destroyed by incubating it with histaminase, the original properties of the toxin remain unimpaired."

From our study it would seem that the toxin injures the epithelial and endothelial cells of the glomerular tufts and the epithelial cells of the convoluted tubules and of the loops of Henle. Any degree of injury may be found from simple cloudy swelling to complete cellular disintegration. We cannot be sure what portion of the tubule is injured to the greatest extent; however, in some of the microscopic sections it is thought that the convoluted portion of the tubule shows the greatest destruction. In the early stage the capillary loops of the tufts are dilated and filled with red blood cells. Later, the capillaries contain only a few red blood cells. In the early process the endothelial cells of the tufts are swollen and only a few of these cells are destroyed. It would seem from this observation that the endothelial cells are more resistant to the action of the toxin than are the epithelial cells that line the tubules. A few of the glomeruli are severely injured by the toxin, as shown by the presence of

desquamated cells, leukocytes and red blood cells in the capsular space, and also by the presence of adhesions between the tuft and the capsule (Figs. 9 and 10) and thrombi in a few capillary loops.

All of the rabbits that died showed an elevation in the non-protein nitrogen; however, there is a wide variation in the total amount of nitrogenous products retained in the blood at the time of death. The retention of the non-protein nitrogen in the blood does not exactly parallel the extent of the renal lesion in all the rabbits. Those animals in which extensive kidney lesions are found show a significant elevation in the non-protein nitrogen and whenever extensive lesions are absent the non-protein nitrogen is within normal limits.

It is of interest to note the relation between the uric acid content of the blood and the retention of non-protein nitrogen. When the non-protein nitrogen increases to 80 to 90 mg. per cent the uric acid does not show any elevation. The normal non-protein nitrogen content of the blood is 30 to 45 mg. per cent and the normal uric acid level is 1 to 1.8 mg. per cent in this group of rabbits.

The lesions produced in the kidney after the intravenous injection of this toxin cannot be considered a true nephrosis, as defined by Fahr, on account of the damage to the glomerulus and of the retention of nitrogenous products in the blood.

SUMMARY

1. A filtrable toxin from a hemolytic *Staphylococcus aureus* produces damages to the tubular epithelium and the glomerulus when injected intravenously into normal rabbits.

2. The most conspicuous lesion occurs in the tubules.

3. The glomerulus is damaged, as shown by the presence of albumin, desquamated cells and red blood cells in the capsular spaces, by the presence of adhesions between the tuft and capsule of other glomeruli, and hyaline thrombi in a few capillary loops.

4. There is a retention of nitrogenous products in the blood of rabbits receiving staphylococcal toxin intravenously.

5. There is no retention of nitrogenous products and the kidneys are normal in control rabbits that receive intravenous injections of sterile peptone broth.

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DESCRIPTION OF PLATES

PLATE 108

- FIG. 1. The capillary loops of the glomeruli are dilated and filled with red blood cells. This rabbit received 0.1 cc. of the staphylococcal toxin subcutaneously and 24 hours later 0.5 cc. intravenously. Death occurred 4 hours after the second injection. Hematoxylin and eosin stain. $\times 200$.
- FIG. 2. Same as Fig. 1. Hematoxylin and eosin stain. $\times 550$.
- FIG. 3. The left kidney in this rabbit was removed 6 days after the intravenous injection of 0.5 cc. of the toxin. The necrosis of the tubular epithelium is focal in distribution. Hematoxylin and eosin stain. $\times 250$.
- FIG. 4. The right kidney of a rabbit receiving intravenously 0.5 cc. of the toxin (the left kidney is shown in Fig. 3). This animal was killed 19 days after receiving the toxin. There are no focal areas of tubular necrosis, as found in the opposite kidney. The débris in the lumen of the tubules is often found in normal rabbits. Hematoxylin and eosin stain. $\times 250$.

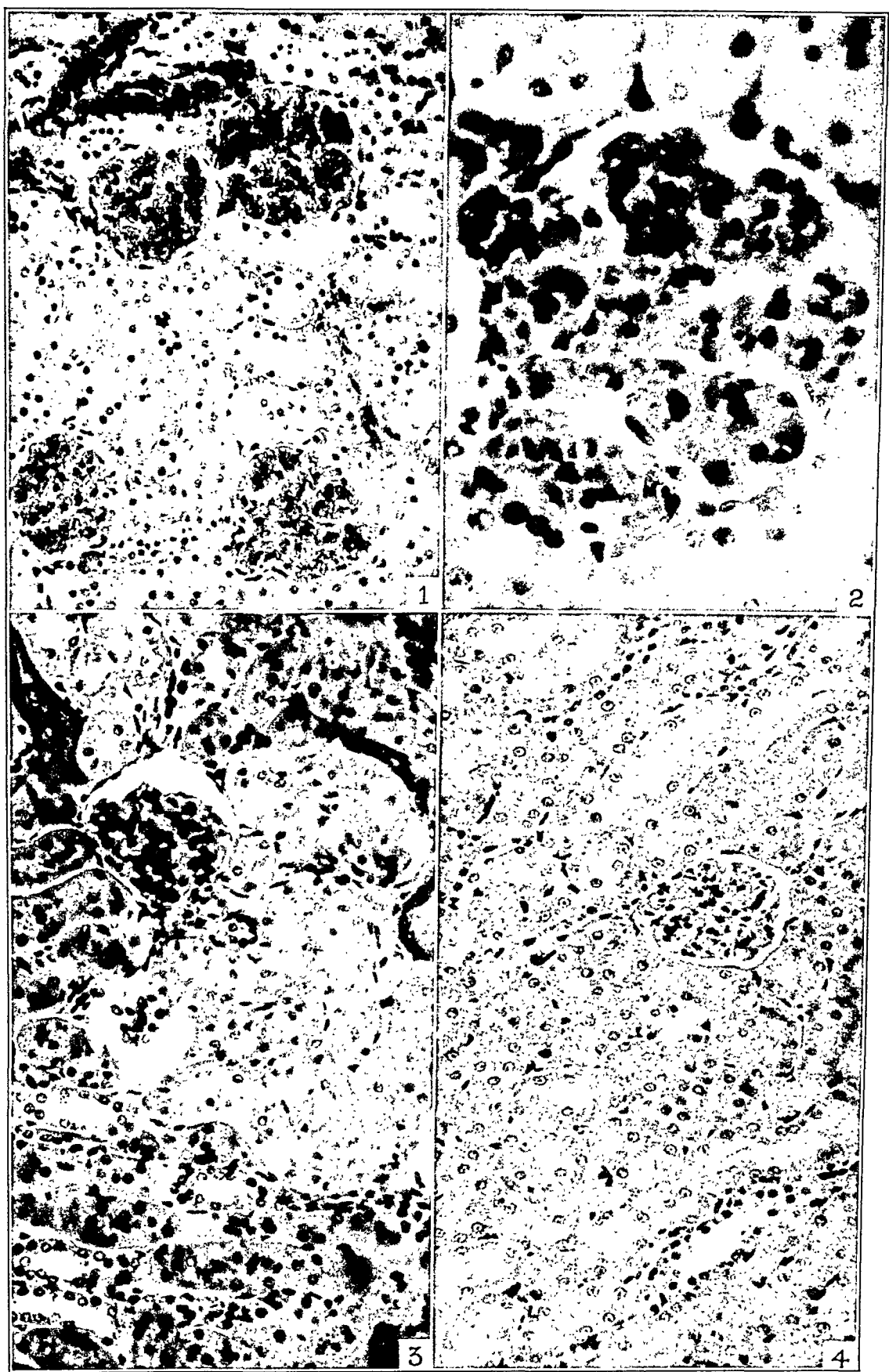
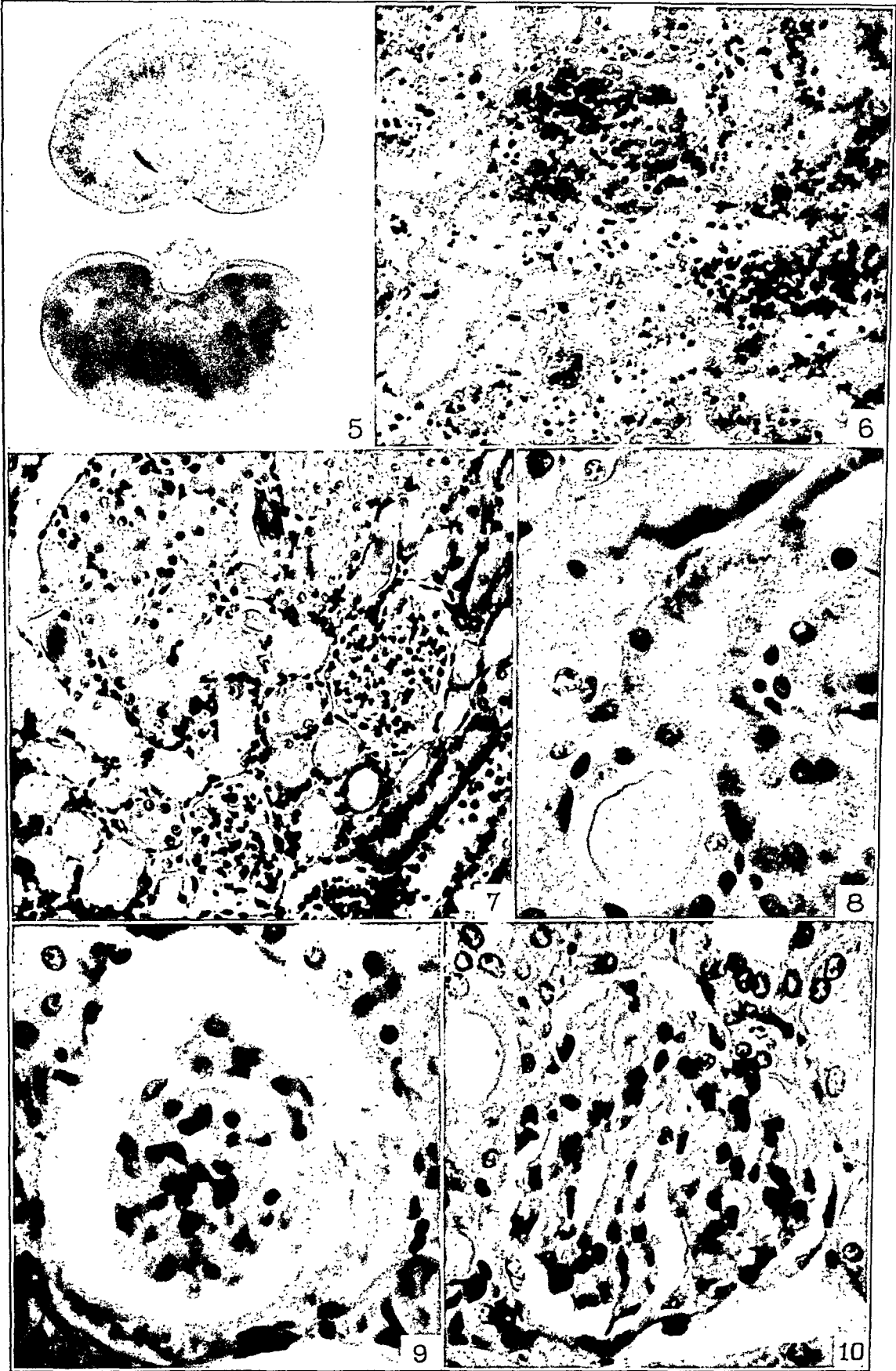


PLATE 109

- FIG. 5. Cortical necrosis and focal hemorrhagic areas in the kidney of a rabbit that received intravenously 0.5 cc. of the toxin and 24 hours later 0.75 cc. of the toxin. Death occurred 37 hours after the second injection.
- FIG. 6. Rabbit 2, (see Table I). The tubular epithelium and the glomeruli are necrotic and polymorphonuclear leukocytes are infiltrating the interstitial tissue of this zone. Hematoxylin and eosin stain. $\times 250$.
- FIG. 7. Rabbit 1, (see Table I). The tubular epithelium is often completely destroyed and the lumens of the tubules are filled with hyaline casts. Hematoxylin and eosin stain. $\times 200$.
- FIG. 8. Same as Fig. 7. Hyaline droplets are present in the epithelial cells and hyaline casts fill some of the tubules. Many of the epithelial cells show these hyaline droplets in the kidneys which have been severely damaged by the toxin. Hematoxylin and eosin stain. $\times 550$.
- FIGS. 9 and 10. Rabbit 3, (see Table I). A few of the glomeruli have red blood cells in the capsular spaces. Other glomeruli show adhesions between the tuft and Bowman's capsule. Hematoxylin and eosin stain. $\times 550$.



A HISTOLOGICAL STUDY OF THE ADRENAL CORTEX IN MONGOLISM *

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The etiology and pathogenesis of mongolism are still unknown. Since the condition was described by Down¹ in 1866 numerous hypotheses have been offered, and among these there may be mentioned syphilis, age of the parents, number of the pregnancy in mother's child-bearing history, alcoholism of either parent and exhaustion of the germ plasm. None of these has successfully withstood critical examination. A discussion of the most recent literature and theoretical considerations is given by Eley,² who concludes that the etiology of mongolism is still obscure.

The latest conjectures, as would be expected, have come from the field of endocrinology, with prominent mention given the pituitary, thyroid, and more recently the adrenal cortex. Because of certain evidence to be discussed it was thought that a study of the adrenal cortex by histological methods might yield data that would be of aid in evaluating the rôle of the adrenal cortex in the production of mongolism. We are not unmindful of the complex and still confused interrelations of the various parts of the endocrine system, nor do we forget the limitations of an examination of merely one organ in the study of a disease of obscure etiology. The purpose of this paper is to determine if there is any histological evidence in support of the theory that the adrenal cortex is in any way involved in the etiology and pathogenesis of mongolism.

THEORETICAL CONSIDERATIONS

Mongolism is as definite a clinical entity as cretinism. Definite stigmata are associated with the disease and a diagnosis can be made almost always at birth. That no basic similarity to cretinism exists has been shown by Talbot,³ who proved by studies of the

* Received for publication January 22, 1934.

basal metabolism of mongols that "the thyroid is not involved in the majority of cases, and then only to a minor degree."

The task of relating any anatomical finding in the adrenal cortex to a hormone produced by the cortex will be a difficult one, for it is probable that the adrenal cortex produces more than one hormone. The work of Hartman and co-workers,⁴ and Swingle and Pfiffner⁵ on the hormone cortin, which prevents death from acute adrenal insufficiency, has been accorded general recognition. There is no suggestion of acute adrenal insufficiency in mongolism. If there is a lack in the adrenal cortex there must exist either a sublethal insufficiency, or insufficiency of a non-vital function of the gland. This latter consideration is probable in view of the possible multiplicity of adrenal cortex hormones.

A discussion of the relation of the adrenal cortex to the development of the central nervous system yields suggestions of value. A lack of cerebral development is an obvious feature of mongolism. That the adrenals may be absent or extremely hypoplastic in anencephalic or hemicephalic monsters has been well known since the first observation in 1842 by Johann Friedrich Meckel. Seventeen instances of this finding were reported by Lomer.⁶ Weigert⁷ and Zander⁸ emphasized that the cortex of the adrenal was primarily affected in anencephaly.

Diffuse amyelination of the cerebrum has been described by Davidoff⁹ in mongols. This finding is of interest in regard to the possible rôle played by the adrenals in myelination of the nervous system. Evidence for this has been adduced from the large size of the adrenal gland during the latter part of gestation at the time when cerebral development is said to proceed most rapidly, and the large amounts of cholesterol in the adrenal cortex during the first year of life, when the greatest activity in the myelination of the central nervous system is noted.

The marked lack of sexual development has long been observed in mongols, particularly in the males, and suggests the possibility of an adrenal cortex insufficiency. Though the relation of the adrenal cortex to sexual development is still a question, there are numerous examples of "adrenal virilism" with precocious sexual maturity and masculinization of females associated with tumors of the adrenal cortex.

A common clinical observation in mongols is the marked hyper-

flexibility and decreased muscle tonus. The decreased muscle tonus in Addison's disease, which is of unquestionable adrenal etiology, is a well known part of that disease picture. In addition, in common with patients suffering from Addison's disease, mongols tend to have low blood pressure and vagotonia.

Although no definite proof has been offered of any direct relation between the adrenal cortex and mongolism, these theoretical considerations do form a fair basis for the assumption that a hypofunction of the adrenal cortex is in some way related to the development of the condition known as mongolism, and justify an attempt by morphological means to test that assumption.

MATERIALS AND METHODS

These studies are entirely morphological in character and are based on observations and measurements of a series of microscopic preparations from the adrenal glands of mongols. A series of 15 cases was assembled from the postmortem files of the Children's Hospital of Boston and all available data relating to the patients were studied. The diagnosis of mongolism was taken from the clinical diagnosis and only unquestionable instances were included in this series. All microscopic preparations were obtained from Zenker-fixed material and were stained by hematoxylin and eosin.

The essential procedure in this study has been the measurement of the width of the permanent adrenal cortex, as seen in cross-section. Measurements of the fetal cortex were also taken. These measurements were made with a micrometer ocular calibrated against a slide with a measured scale. Certain criteria of a true cross-section (*e.g.* cut in a plane perpendicular to the surface of the gland) were applied in an effort to obtain comparable data. Measurements were made only of those portions of the cortex in which the two opposite layers were approximately parallel to each other. For that reason the apex of a leaflet of the gland was not chosen (Fig. 1). Furthermore, only such places were measured where there was a full length longitudinal section of the fasciculata, which showed termination of the columns in a solid line rather than in a series of uneven blocks, as is found in sections that have been cut obliquely (Fig. 2). It was also endeavored to select areas for measurement where the two opposite layers of cortex were approximately the same width (Fig. 1).

Measurements were also made on a series of suitable controls, as will be indicated below. In the evaluation of these measurements some difficulty was encountered in arranging the series in order of relative maturity of the patients at the time of death. Because mongols lag behind normal children in development and because it is impossible to rule out the factor of prematurity, which would have an effect upon the actual age of the individual, it was decided to arrange the findings in two ways — by body length and by chronological age. It was not possible to obtain enough material for controls to match the mongol series by cause of death and sex, as well as age and body length, but these factors were taken into account wherever possible.

In most cases ten measurements were taken along the cross-section of the permanent cortexes satisfying the above criteria. If, however, the deviation of any one measurement from the mean of the ten measurements was more than one-third of the mean, a second set of ten measurements was taken. If the mean of the second ten differed from the mean of the first ten by more than one-third of the mean of the two sets the slide was rejected as being unsuitable for measurement. Consequently, the means recorded may be considered as statistically reliable measurements of the widths of the respective permanent cortexes within the limitations inherent in the method itself. Whenever the difference between the mean of the second ten measurements and the mean of the first ten was found to be within one-third of the mean of the two, the slide was retained and the probable error was calculated on a basis of twenty measurements representing the sum of the two sets.

Whenever possible the width of the fetal cortex was measured, as well as that of the permanent cortex, and the measurements were accorded the same statistical treatment as those of the permanent cortex. In many sections the fetal cortex was represented by scattered groups of cells, making measurement of its width impossible.

The measurements recorded in the tables were all made on fixed material. As no allowance was made for shrinkage by fixation, they do not represent a measurement of the absolute width of the cortex, but are probably somewhat less than the actual width in the fresh gland.

After arranging the means of the widths of the permanent cortexes of the mongols and the controls in body length and chrono-

logical age groups the arithmetic mean of each group was obtained for the purpose of comparing the means of widths of the permanent cortices of the mongol groups with those of the respective control groups. These means will be found recorded in tabular form below.

TABLE I
Means of Body Length Groups

Mongols			Controls		
Group	No. cases	Mean of permanent cortex	Group	No. cases	Mean of permanent cortex
1 46-47 cm.	4	0.355	45-48 cm.	16	0.35
2 51-52 cm.	2	0.38	50-53 cm.	8	0.45
3 58-65 cm.	6	0.43	57-66 cm.	24	0.47
4 68-70 cm.	2	0.42	67-73 cm.	8	0.52
5 about 80-85 cm. ...	1	0.47	75-90 cm.	4	0.60

TABLE II
Means of Age Groups

Mongols			Controls		
Age groups	No. cases	Mean permanent cortex	Age groups	No. cases	Mean permanent cortex
1 7 mos. prenatal ... 2½ mos. postnatal to full term 3 wks.	5	0.36	7½ mos. prenatal ... 15 hours to full term 1 month	20	0.375
2 4½ mos. to 7 mos. .	5	0.40	4 mos. to 7 mos.	12	0.46
3 9 mos. to 11 mos. .	4	0.45	8 mos. to 12 mos. ...	10	0.525
4 2 to 4/12 yrs.	1	0.47	2 yrs. to 2 8/12 yrs. ...	4	0.605

RESULTS OF PRESENT STUDY AND DISCUSSION

A microscopic study of the adrenal glands of mongols reveals no constant pathological findings whereby a diagnosis of mongolism can be made in this manner. Our tables of measurements of the adrenal cortex show that the amount of variation among the members of various groups of the same age or body length is considerable. However, when the means obtained from the mongol group are compared with the means of the control groups it can be seen that:

1. The width of the permanent cortex of mongols in the first year of life does not differ essentially from that of controls of the same age.
2. There is a definite lag in the growth of the permanent cortex in the older mongols, as compared with the control group.

The magnitude of the difference is indicated in the tables. It can be shown from our study that there are individual cases in which the permanent cortex of the mongols is actually greater than some of the individuals of the same body length or age in the control group. The lack of a constant difference between the measurement of the permanent cortex of any given mongol and the control is a finding of great importance in evaluating the results of the present study. Since in the series the permanent cortex of several mongols was actually equivalent to, or greater than, members of the control group, the primacy of the adrenal cortex in the etiology of mongolism is definitely precluded. The difference in the means as maturity advances is, however, also an important finding in giving an indication that a tendency toward hypoplasia of the adrenal cortex is a part of the pathological picture of mongols. That this hypoplasia is real and not to be explained by the fact that mongols lag behind normal children in physical development is shown by the similarity of result by both the age and body length grouping. The body length arrangement controls the factor of physical retardation.

Speculation about the cause of the tendency toward hypoplasia of the permanent cortex of the adrenal gland in mongols is entirely beyond the scope of this paper. Although the primacy of the adrenal gland in the etiology of mongols appears to be ruled out by this study the hypoplasia of the adrenal cortex, though secondary to some primary cause, may well explain certain less essential manifestations of mongolism (the hyperflexibility and the lack of sexual development). If, as has been suggested by Macklin,¹⁰ mongolism is hereditary and is due to the coming together of several recessive characters, the hypoplastic adrenal cortex may be one of the factors involved in a pleuriglandular endocrine insufficiency explained on such a genetic basis.

SUMMARY

1. A method for measuring comparable widths of the adrenal cortex is described.

2. The lack of any characteristic pathological picture in the adrenal cortex in mongolism is noted.

3. The results of a measurement of the adrenal cortexes in a series of 15 mongols compared with 60 controls are tabulated and arranged in chronological age and body length groups. According to our measurements:

- (a) There is considerable individual variation in the measured widths of permanent and fetal cortexes among the members of groups of similar age or body length of both the mongols and the controls.
- (b) There are individual cases in which the permanent cortex of a mongol is actually greater than some individual controls in the same age or body length group.
- (c) There is a definite retardation in the development of the width of the permanent cortex of the mongol adrenals so that there is an actual hypoplasia of adrenal cortex of older mongols, as compared with the controls.

CONCLUSIONS

As maturity advances, a definite hypoplasia of the adrenal cortex in mongols becomes evident by the use of histological methods in the measurement of the width of the permanent cortex of the adrenal gland.

We wish to thank Miss Marjorie T. Bellows, statistician, Westchester County Health Department, New York, for assistance in computing the probable error in the study. Prof. E. B. Wilson of Harvard University kindly pointed out the statistical requirements for this study.

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DESCRIPTION OF PLATE

PLATE 110

FIG. 1. Photomicrograph showing typical cross-section of apex of leaflet of adrenal gland, satisfying criteria of true cross-section; *i.e.*, full length longitudinal section of fasciculata terminating in solid columns (see text).

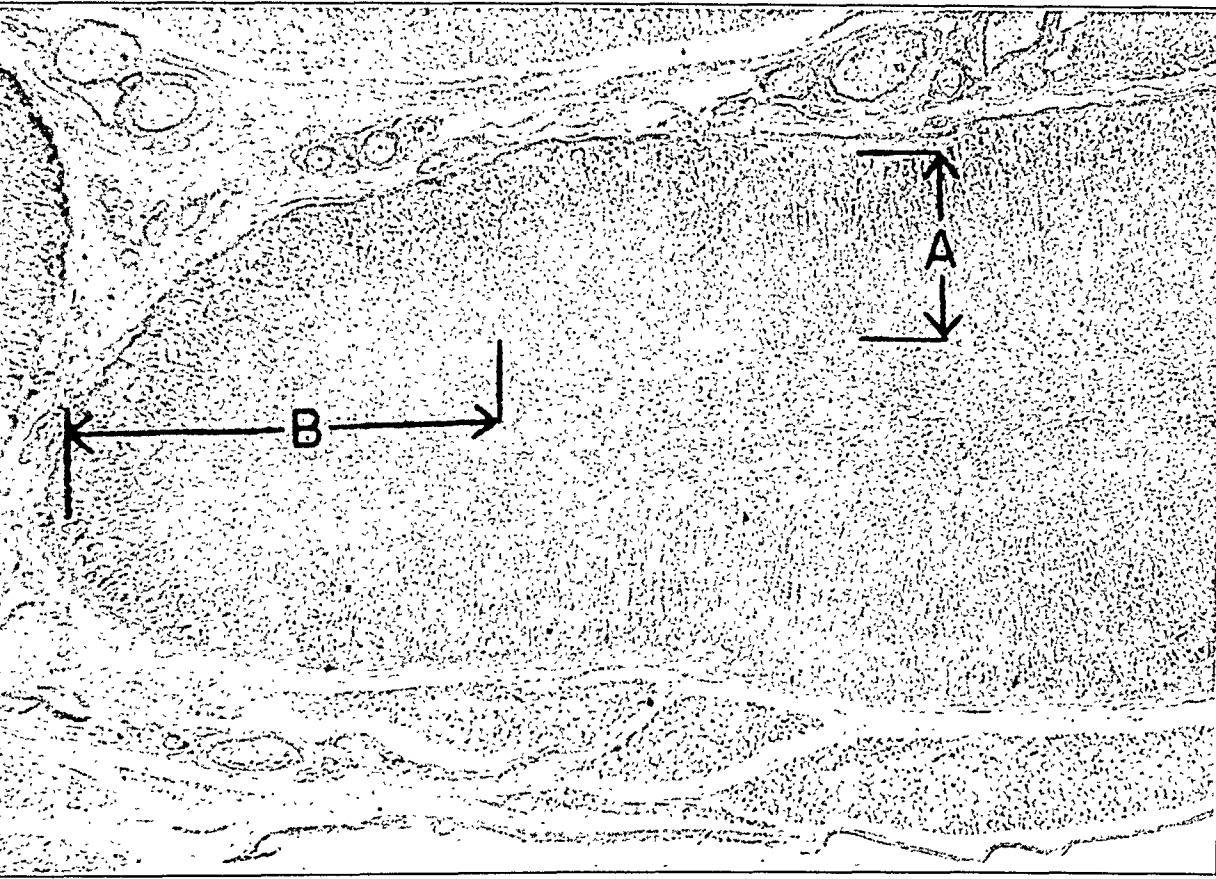
A = typical site of measurement for width of cortex with opposite layers of cortex approximately parallel.

B = width through apex not used as site of measurement. Hematoxylin-eosin stain. $\times 30$.

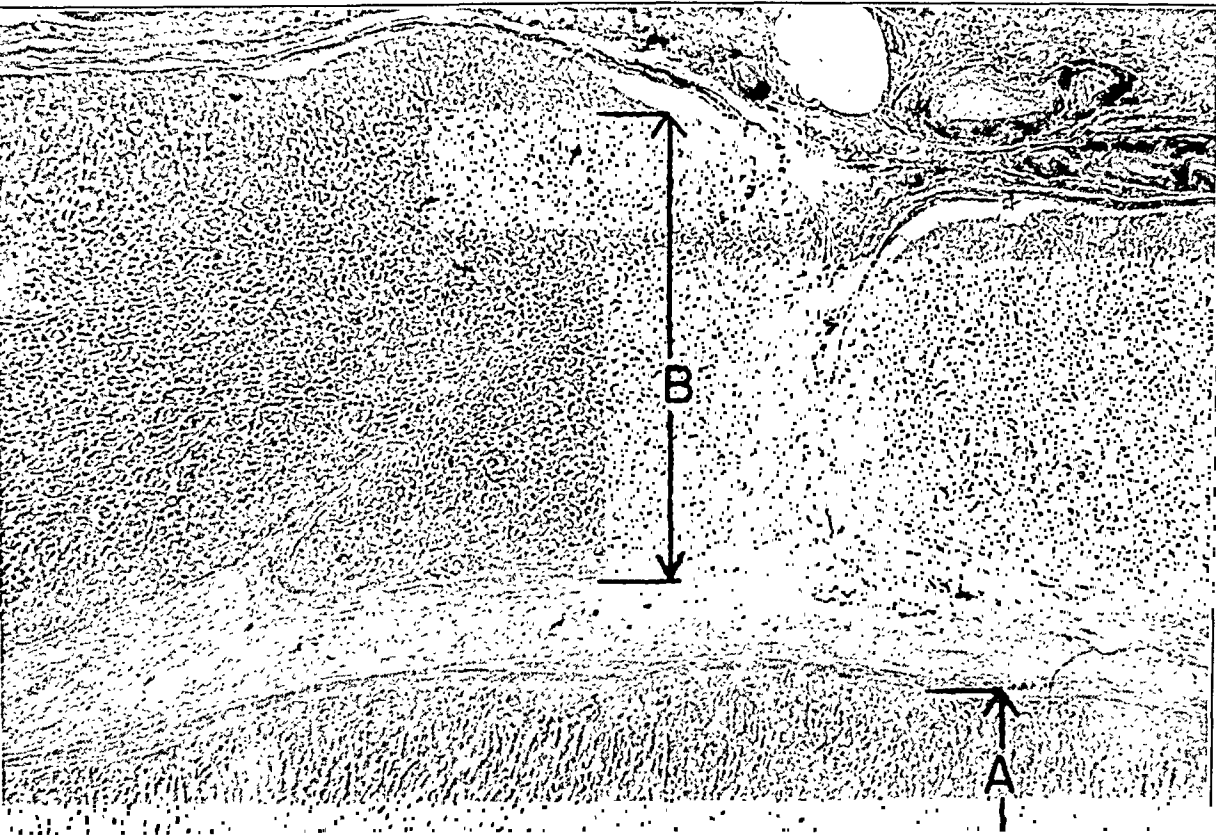
FIG. 2. Photomicrograph showing example of oblique and coronal section of the type that was rejected as being unsuited for measurement.

A = oblique section of cortex.

B = coronal section of cortex. Hematoxylin-eosin stain. $\times 30$.



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THE AMERICAN JOURNAL OF PATHOLOGY

VOLUME X

JULY, 1934

NUMBER 4

TUMORS AND TUMOR-LIKE CONDITIONS OF THE LYMPHOCYTE, THE MYELOCYTE, THE ERYTHROCYTE AND THE RETICULUM CELL *

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During the seven years that the Lymphatic Tumor Registry ‡ of the American Association of Pathologists and Bacteriologists has been operating 380 cases have been contributed. Fifty of these cases were not tumor or tumor-like conditions of either lymphatic, hemopoietic, or reticulo-endothelial tissues. Of the balance all are of interest and value in the study of these conditions, but only about half of the total number have sufficiently complete records and material to permit the defining of the conditions present. Therefore, too few cases have as yet been contributed to the Registry to make statistical study of the individual conditions of much value in determining the age distribution, clinical character, course and outcome of each type of lesion. The cases most valuable to the Registry are those that have been followed to their conclusion, autopsy performed, and material furnished from biopsy and autopsy.

All cases in the Registry have been reviewed each year by the Registrar preliminary to rendering the annual report. All cases offering definite difficulties in diagnosis, in which adequate data and ma-

* Received for publication February 13, 1934.

† Registrar, American Registry of Pathology.

‡ The Lymphatic Tumor Registry of the American Association of Pathologists and Bacteriologists was established in 1925. It is now the Lymphatic Tumor Division of the American Registry of Pathology maintained at the Army Medical Museum under the auspices of the National Research Council. The committee for the Association consists of Dr. F. B. Mallory and Dr. James Ewing, to whom all difficult cases are referred. The author is Registrar and here expresses his deep appreciation for the invaluable coöperation of the committee, but assumes the entire responsibility for the opinions herein expressed.

terial were available, have been referred to the consulting committee and often to other pathologists. As a result of these studies points of importance in differential diagnosis have been brought out and it is believed that their presentation at this time may stimulate thought and discussion to the end that we may arrive at a classification of the tumor and tumor-like conditions of the lymphatic, hemopoietic and reticular tissues.

The nomenclature of these conditions in the textbooks and articles in periodicals is extremely variable, and there is a like variation in the nomenclature in the diagnoses received from the committee and from others who have examined the material. As is true of tumors of any structure there are many atypical cases, but there is little agreement in terminology. The data and illustrations in the literature are rarely adequate to define the entity presented, so that published reports have been found of little value in establishing a standard nomenclature. The cases forming the basis of this report are not all complete but were selected as conforming to types of which there are a sufficient number to be of value in classification.

In this discussion it is not considered wise to enter into the controversy concerning the ultimate origin of the cells concerned, but rather to consider the adult cell and attempt to define the conditions that arise from it. (For this purpose the following stem cells each form a group and each group is subdivided into the types that appear to be represented in the Registry collection. These are the stem cells giving rise to the *lymphocyte*, the *polymorphonuclear leukocyte*, the *red blood corpuscle*, and the *monocyte* or reticulo-endothelial cell. It is realized that exception will be taken to the inference that the monocyte and the reticulo-endothelial cell are one and the same or that they have a common origin, but these neoplastic conditions present certain indications that such is the case.

It is not believed that there are any concepts in this article that have not been presented or at least suggested before. To trace these for priority of publication is beyond the energy of the writer and the scope of this article.

LYMPHOCYTE

I. Lymphocytosis: Lymphoma

Unless the term *lymphoma* is applied to inflammatory reactions there are no cases in the Registry.

TABLE I
Classification of Tumors and Tumor-like Conditions of the Lymphatic, Hemopoietic and Reticular Tissues

Adult cell type	Lymphocyte	Myelogenous		Reticulum cell	
		Granular leukocytes	Red blood corpuscles	Reticulocyte monocyte	Hodgkin's disease
I† Reactions	**"Lymphoma" Lymphocytosis	Leukocytosis	Symptomatic polycythemia	*Gaucher's disease Niemann-Pick disease	Localized (sclerosing)
II Proliferations of neoplastic type	Leukemic lymphocytoma 1. Chronic 2. Acute	Leukemic myelocytoma 1. Chronic 2. Acute	1. Polycythemia vera (Syn. Erythremia) 2. Leukemic erythrocytoma	Leukemic reticulocytoma (Syn. Monocytic leukemia)	←
III	Aleukemic lymphocytoma 1. Diffuse 2. Nodular	Aleukemic myelocytoma 1. Single 2. Multiple (Syn. Multiple myeloma)	*Aleukemic erythrocytoma	Aleukemic reticulocytoma	Generalized (cellular)
IV Malignant tumors	Lymphosarcoma 1. Aleukemic 2. Leukemic (Syn. Lymphatic leukosarcoma)	Myelosarcoma *1. Aleukemic 2. Leukemic (Syn. Myelocytic leukosarcoma) Chloroma	Erythrosarcoma *1. Aleukemic 2. Leukemic	Reticulum cell sarcoma	Sarcomatous

* Type not observed in Registry.

† Roman numerals refer to text heading.

II. *Lymphocytoma, Leukemic — Syn. Lymphatic Leukemia*

1. Definite leukemia, 25,000 or more white blood cells with a preponderance of lymphocytes. The number of cells varies in the individual case and from time to time in the same case. Also, cases that primarily are classified in the aleukemic group (below) may become leukemic and remain so, either after irradiation or spontaneously. The type of cells may be uniform or considerable variation may occur. The more numerous the younger forms, the more rapid the course of the disease and usually the younger the patient.

2. The enlargement of lymph nodes is usually generalized and the spleen, or liver, or both, usually are more or less increased in size. There may be an area of greater enlargement, especially in those cases in which the enlargement is the first symptom noted. This is true of those cases of aleukemic lymphocytoma that later become frankly leukemic. In general, the greater the number of lymphocytes in the blood the less the swelling of lymph nodes, and *vice versa*.

3. Microscopically there is an increase in the size of the primary nodules, due to proliferation of the lymphocytes. This proliferation finally obliterates the node structure so that it becomes a uniform mass of lymphocytes. In the liver the portal spaces are distended or enlarged by lymphocytes which also infiltrate between the liver cords. In the spleen the malpighian nodules are enlarged and encroach on the red pulp, often apparently obliterating it. In other organs diffuse infiltrations occur but these, even when grossly visible as pale areas, appear to be proliferations starting in preëxisting collections of lymphocytes. In this condition there are no true metastases. These infiltrations are more frequent and larger in the acute forms of leukemia. Some cases show many atypical cells and then approach the sarcoma type. Occasionally, cases primarily leukemic terminate in typical metastatic lymphosarcoma.

4. The reticulum of the nodes is not increased but is distended or separated by the lymphocytic increase.

5. Irradiation is effective in reducing the size of the tumors and the number of cells in the circulating blood. In many cases it appears to have increased the duration of life but it is only a palliative treatment and not curative.

III. Lymphocytoma, Aleukemic — Syn. Lymphatic Pseudo-leukemia

(a) Diffuse Type:

1. Leukemia is absent but there are always abnormal lymphocytes in the blood if proper search is made for them. At times, and often throughout the course of the disease, there is an actual increase in lymphocytes. It is difficult to make a dividing line between the aleukemic and leukemic forms, as these merge into one another and the aleukemic form may become leukemic and, rarely, cases which when first observed have a leukemia of 25,000 or more may become aleukemic. For the purpose of this classification 25,000 or more white blood cells, with a preponderance of lymphocytes, have been considered necessary for the diagnosis of leukemia.

2. The swelling of the lymph nodes is less generalized than in the leukemic form and there is usually a region of greatest intensity. The spleen, or liver, or both may participate in the process.

3. Histologically the picture in the nodes may be the same as in the leukemic form, or a group of nodules may coalesce and leave some relatively unchanged node structure. This is especially true of the more rapid, fatal processes at the younger ages in which the cells often appear to be of unusually large size and show relatively frequent mitoses. Infiltrations are unusual and when present indicate an approach to leukemia or the presence of a leukemic change unrecognized before. It is not unusual to find evidence of a definite leukemia at autopsy, which had not been discovered in blood examinations. It represents a change subsequent to the blood examination last recorded and may have taken place within a few days of death. When atypical cells or many mitoses are found the condition approaches that of a lymphosarcoma which is sometimes the terminal picture in this group.

4. Reticulum the same as in the leukemic form.

5. Irradiation is effective in reducing the size of the nodes as a palliative measure. Following X-ray these cases may become leukemic.

(b) Nodular Type — Syn. Giant Follicular Hyperplasia with Splenomegaly:

1. The blood changes are similar to those in the diffuse form (above) but apparently leukemia develops less frequently.

2. The swelling of the lymph nodes is the same as in the diffuse form and is sometimes quite generalized. The spleen has always been involved, and in two observed cases was apparently the only focus of the disease.

3. Histologically the condition is characterized by large nodules, which appear to be of a secondary type, and an apparent increase in their number. The nodules are composed of the large type of lymphocytes, among which are rather numerous mitotic figures. The nodules may be contiguous and rarely appear to have coalesced, though there is often marked variation in size in the same section. These nodules are surrounded by relatively normal lymphocytes of the smaller size.

These individuals either die rather quickly or, if life is prolonged, the process terminates in a definite sarcomatous change in which there are metastases. In this type the cells show more pleomorphism than in any other lymphocytic tumor, resembling to some degree the cell picture of sarcomatous Hodgkin's disease, with which the condition occasionally has been confused.

4. Reticulum is loose meshed as in the diffuse form, thus differing from Hodgkin's disease or Hodgkin's sarcoma (see below).¹

5. This condition is very sensitive to irradiation which, properly used, will prolong life for many years. Superficial nodes well radiated may never swell again. It is quite possible that this condition may be curable when localized and treated early by adequate irradiation.

IV. *Lymphosarcoma*

(a) *Aleukemic:*

1. Typically, there are no abnormal findings in the blood, but following irradiation this form may become leukemic (see (b) below).

2. Tumors are localized and infiltrate, metastasize, or both. Metastatic nodules are found in situations where usually lymphocytic groups do not occur, as in the intermediate zone of the liver, the lower cortex of the kidney and the heart muscle.

3. Histologically the tumor itself is made up of atypical lymphocytic types with irregular nuclei and scanty cytoplasm. Mitoses are usually abundant. The more atypical the cells, the more malignant the tumor. Following irradiation the cells may become more typical.

4. Reticulum is loose meshed, as in the preceding types.

5. Very sensitive to irradiation, so much so that it is possible that taken early and adequately treated the tumor may be cured.

(b) *Leukemic — Syn. Lymphatic Leukosarcoma:*

1. Leukemic blood with a considerable proportion of abnormal young or embryonal types of lymphocytes.¹ The leukemic condition in lymphosarcoma may be spontaneous, may follow irradiation, or a leukemia may terminate in a sarcomatous spread.

2. Local tumor mass invading, metastasizing, or both, with metastatic nodules as in the aleukemic form. In addition gross infiltrative types of spread may be found, as in ordinary leukemia.

3. Histologically the cells are atypical, as in the aleukemic form. In those cases that are primarily aleukemic but become leukemic after irradiation the cells become more typical.

4. Reticulum as in the other lymphocytic groups above.

5. Sensitive to irradiation to some degree but many cases terminate so quickly that no adequate data have been accumulated. The cases that have become leukemic after irradiation appear to be less sensitive than the aleukemic form and it is possible that the leukemic change in these is the result of inadequate or improper irradiation dosage.

The group of conditions arising from the lymphocyte or its stem cell is the best defined and best understood of all the groups here considered. The term lymphoblastoma may be applied to the entire group, but the different members must be otherwise designated as the term does not sufficiently define the entities. As there are no benign, non-inflammatory lymphomas in the Registry the criteria have been omitted but the condition has been placed, together with lymphocytosis, in the reactive group. Lymphocytosis is represented by several cases of glandular fever, the cell picture of which is that of a lymphocytic proliferation with active hyperplasia of the secondary nodules.

The rest of the group is familiar to most, under a variety of names which sometimes vary because of minor cytological or clinical differences, though more often nomenclature depends on previous instruction, either undergraduate or postgraduate. The simplest and shortest terms have been preferred in this work and the prefix "a" is sufficient to differentiate the leukemias from those conditions that are quite similar except for the absence of an appreciable increase of

cells in the peripheral blood. The term pseudoleukemia, also, is quite acceptable as accurately descriptive. The term "aleukemic leukemia" certainly has nothing to recommend it, even though there are phases in leukemias during which the leukemia is diminished or absent. Such phases, whether the result of treatment or not, can be designated as such without the use of a name suggesting a change to a different disease.

In this group arising from the lymphocyte there is considerable shifting from one subdivision to another, both spontaneously and as a result of irradiation. For instance a leukemia may terminate in a lymphosarcoma, or may become aleukemic. Leukemic or aleukemic lymphocytoma may become sarcomatous. In all aleukemic cases in the Registry from which blood films have been furnished abnormal lymphocyte types have been found, while frequently during the course of the disease a definite lymphocytosis is present even though a frank leukemia never appears.

Lymphosarcoma usually appears and continues as such. Occasionally, as indicated by the literature, a combination of a metastasizing tumor and a leukemia occurs (leukosarcoma) and may be the condition discovered at primary examination! In the Registry material a condition of leukemia has followed irradiation of lymphosarcoma in six instances and in these the progress of the disease has appeared to have been retarded as a result of the change.

The liver is an important organ to study in the differentiation of types. In the lymphocyte group there is a general proliferation of the lymphocytes in the portal areas, increasing the size of these areas either with or without infiltration between the liver cords. The infiltration is present in leukemia and this finding at autopsy always suggests that whatever may have been the findings in the blood a leukemic condition had existed perhaps only near the termination of the illness.

(In this classification the extension of the process to other nodes or lymphocytic tissues is not considered a metastasis.) Whether this spread is the result of a stimulus in the circulating blood or whether it is due to cells from the original process reaching the new areas by blood and lymph, and there only finding satisfactory conditions for growth, is not known. The latter alternative seems the more probable method.))

GRANULAR LEUKOCYTE

*I. Leukocytosis of the Granular Leukocytes**II. Myelocytoma, Leukemic — Syn. Myelogenous Leukemia*

1. Definite leukemia, 25,000 or more white blood cells with a large proportion of myelocytes. The number of cells in the blood varies over a rather wide range even in a single case, and there is also a variation in the relative proportions of the different myelocyte types. In some cases, with or without treatment, the number of cells may decrease to a very low total, though more often the reverse is true. The greater the variation in cell type, usually, the more rapid the course of the disease.

2. The bone marrow shows marked myelogenetic activity and, therefore, there is a decrease in the red marrow. The spleen is markedly enlarged in most cases and the nodular markings are obscured. The liver is also enlarged to varying degrees. Lymph nodes usually do not participate in the swelling. Sometimes infiltration deposits are visible grossly in the tissues.

3. Microscopically there is marked myelogenetic activity in the bone marrow. The proliferating cells replace the marrow fat and crowd the erythrocytic tissue. There is no bone destruction. In the spleen myelogenesis is usually active and takes place in the red pulp. It crowds and compresses the malpighian nodules and practically obliterates some of them. In the liver there is a diffuse infiltration, usually somewhat more marked in and near the portal connective tissue, but there is no great enlargement of these areas. Infiltration is more or less generalized and often relatively large masses are formed which suggest metastatic tumor. Where lymph nodes appear enlarged the histological picture is that of an infiltration, as in other organs. Occasionally leukemias become definitely sarcomatous and thus would be finally classified in Group IV, below.

4. There is no increase in reticulum in the marrow, though the spleen, especially after irradiation, shows some increase in fibrous tissue.

5. Irradiation is effective as a palliative measure in many cases. In the more acute forms it often appears materially to retard the process.

III. *Myelocytoma, Aleukemic ((a) Single, (b) Multiple) — Syn. Single and Multiple Myeloma*

1. Aleukemic but young cells are usually present in the blood and leukemia may occur during the course of the disease.

2. (a) Single myeloma occurs in long and flat bones where it produces definite tumors of osteolytic type without much tendency to spontaneous fracture.

(b) Multiple osteolytic tumors of the marrow, destroying the bone from within and leading to spontaneous fracture which is often the first definite symptom.

In both single and multiple myeloma there are no true metastases, though the invasiveness of some of them approaches the sarcoma type and sarcomatous changes may occur and metastases take place.

3. Microscopically the cells composing these tumors show considerable variation between different cases but tend to be rather uniform in the individual case. The most uniform picture is that of the so-called plasma cell type which is found in both the single and multiple form. Others show some variation in cell size and staining characteristics. Variation in size is an indication of greater malignancy, an approach to the true sarcoma in which there may be extreme variation in size and form.

4. No reticulum is produced by the tumor cells, though there is some supporting reticular tissue accompanying blood vessels.

5. These tumors are sensitive to irradiation but to what degree cannot be decided by the few cases in the Registry.

IV. *Myelosarcoma*

(a) *Aleukemic:*

This has not been observed in the Registry material. It is possible that this form is represented by those cases of single and multiple myeloma that invade other tissues without metastasis. The metastatic myelogenous neoplasms in the Registry have all been leukemic.

(b) *Leukemic — Syn. Myelocytic Leukosarcoma:*

This group includes the "green" tumor, chloroma.

1. The blood shows a definite leukemia in which there are many embryonal types of cell. The total count varies but is not often as high as in the chronic leukemias.

2. Aside from the bone marrow changes, which may be evenly generalized or have a focus of greater intensity, there are growths from the bones into adjacent tissues and metastatic deposits in parenchymatous organs. The primary spread from the bone, the metastases, or both, may show the green color characteristic of chloroma.

3. The cells are always more varied in form and staining than in the non-sarcomatous type and if a sarcomatous change occurs in a case of simple leukemia there may be found a definite focus of sarcoma type in the bone, surrounded by the usual picture found in simple leukemia.

4. Reticulum is not formed by the tumor cells.

5. Radiation sensitivity not determined by Registry material or literature. Theoretically this group should be sensitive to irradiation, but the course is usually so brief that no adequate data are available.

The stem cell for the granular leukocyte gives rise to the myelogenous, myelocytic leukemias, the aleukemic myelomas and the myelosarcomas or metastasizing myelocytic tumors. This group shows less shifting from one group to another but occasionally a sarcomatous change occurs in a myelogenous leukemia and likewise a leukemia may develop in an aleukemic myeloma. The principal difficulty in this group is the question as to which types are sarcoma. Both single and multiple myelomas destroy bone and by this invasiveness to some degree merit the term of sarcoma; but, the lymphocytomas by pressure may destroy neighboring tissues and the cells invade the repair-like process at the periphery of the growth. When single or multiple myelomas break through the bone and invade surrounding tissues they may be considered as truly malignant and therefore could be considered as belonging to the group of aleukemic myelosarcomas.

Aside from the multiple myelomas the sarcomatous myelocytomas in the Registry have all been leukemic. Chloroma is represented. The green pigmentation is occasionally present in the more malignant myelomas without leukemia.

In the leukemias the infiltrations are difficult to differentiate from true metastases. It is of little practical importance to make this differentiation. Infiltrations are usually more frequent and extensive in the cases clinically more malignant.

In the liver infiltrations are more prominent in and near the portal areas, but masses of considerable size are not found, as in the lymphocytic type. Sometimes lymph nodes show considerable enlargement as a result of infiltrations. Such infiltration is diffuse and the architecture of the node is preserved.

In myelocytic leukemia the blood film often shows considerable numbers of immature red corpuscles and nucleated cells of the "blast" type. This appeared to be compensatory, or at least consistent with the idea that parts of the marrow are forced to overwork and throw out immature forms in the effort to compensate for marrow destruction or replacement by the granular myelocytic proliferation.

RED BLOOD CORPUSCLE

I. Polycythemia, Symptomatic

An increase in the red corpuscles of reaction origin due to some definite and readily determined cause, such as being in high altitudes.

II. (a) Polycythemia Vera — Syn. Erythremia

This condition is suggested for this position as the analogue of chronic types of leukemia.

1. Increase of red corpuscles in the blood. Platelets are increased and, especially in the later stages, there is an increase in granular leukocytes which might be considered as analogous to the appearance of considerable numbers of "blasts" in myelocytic leukemia.

2. The bone marrow shows erythropoietic hyperplasia. The spleen is enlarged, usually with relatively little evidence of erythropoiesis. The enlargement appears to be due to the increase in blood corpuscles in the red pulp.

3. Reticulum of the organs concerned shows no change.

4. Irradiation is apparently of little benefit.

(b) Erythrocytoma, Leukemic — Syn. Erythrocytic Leukemia

This condition, a leukemia of the precursors of the red blood corpuscles, the erythrocytes, is the analogue of the acute leukemias; that is, those in which a large proportion of the cells are of more embryonal type than those normally found in the circulating blood.

1. Definite leukemia, the cells of which resemble lymphocytes, have little cytoplasm, often appearing as naked nuclei. Many "blasts" are present, the larger types predominating. Many abnormal red corpuscles.

2. Bone marrow shows marked erythropoiesis, the yellow marrow being replaced. The spleen is enlarged sometimes to a considerable degree. Lymph nodes are sometimes slightly enlarged, particularly those of the abdomen.

3. Microscopically in the marrow there is a replacement of the fat by embryonal cells of the erythro-genetic group, with an apparent crowding of all normal marrow cells. Even the strands of erythro-genetic tissue seem less numerous than normal. The predominating cell is polygonal with a vesicular nucleus and considerable cytoplasm which is non-granular. The spleen shows a productive type of process in the red pulp, in which erythro-genesis appears to be active. Lymph nodules are crowded by the infiltration or proliferation and some appear to be obliterated. In the lymph nodes there is an infiltration between the lymph nodules and, in this infiltration and in the spleen, megakaryocytes in all stages of development are usually found. These cells in certain stages of development may be confused with the Dorothy Reed cells and a diagnosis of cellular Hodgkin's disease made.

4. No new reticulum is formed.

5. Irradiation sensitivity — no data.

III. Erythrocytoma, Aleukemic

There are no cases of this type in the Registry. The condition of erythroblastic anemia seen in the earlier age groups appears to satisfy the requirements of this position.²

IV. Erythrocytic Sarcoma

(a) Aleukemic:

Not observed in the Registry material. (See note above.)

(b) Leukemic:

This condition was distinguished from the leukemic erythrocytoma by the presence of metastases in the one case that has been observed. These metastases were small hemorrhagic nodules of erythro-genetic tissue.

The group of conditions arising from the stem cell of the red corpuscles is of particular interest because so few cases have been recognized. In this study the writer has used the term erythrocyte to indicate the nucleated form preceding the cell which after loss of its nucleus becomes the corpuscle. Other terms are difficult to adapt, but it is realized that erythrocyte is used as a synonym for red blood corpuscle.

In this group few will disagree with the placing of symptomatic polycythemia in the reactive position. It is also possible that eventually all cases of polycythemia vera, erythremia, can be so placed, but the blood, marrow and spleen in cases of erythremia are analogous in their enlargement and activity to the picture seen in chronic myelocytic leukemia. Careful study of the blood in erythremia occasionally shows blasts, while among the leukocytes are usually found atypical mononuclear cells which cannot be definitely placed in the myelocyte group.

The analogue of acute leukemia in this group is characterized by a leukemia of non-granular cells having little cytoplasm and which cannot readily be differentiated from embryonal lymphocytes. In addition, however, there are numerous "blast" types and many abnormal corpuscles. The bone marrow in general is red, but with pale areas or large involvements of solid tumor-like tissue replacing the normal marrow. In the spleen the change or proliferation is in the red pulp, there being no proliferation of the malpighian nodules.

In the spleen and to some extent in the lymph nodes there is active erythropoiesis, which in the nodes appears as a part of an infiltrative process composed of undifferentiated cells, erythroblasts and a diffuse infiltration of the mature forms, the red corpuscles. Except for the type of cell the picture is that of a myelocytic condition. Hasty examination of the blood film usually leads to a diagnosis of acute lymphatic leukemia. The marked tendency to hemorrhage and the large number of "blasts" in the film are the clues that suggest the correct diagnosis. The formation of typical adult megakaryocytes in the affected tissues is of value but also seems to confuse the condition with Hodgkin's disease. The lack of sclerosis and the infrequency of typical Dorothy Reed types is of assistance in differentiation. In the nodes the fact that the process is infiltrative and does not show proliferation of the lymphocytes of the nodules or the inter-nodular stroma is an important differential point.

THE RETICULOCYTE (MONOCYTE)

Certain reactive conditions show such a preponderance of reticular hyperplasia that they are placed in this group rather than in that of the lymphocyte. These include tuberculosis, leprosy, tularemia and some others. Hodgkin's disease is characterized by a reticular hyperplasia and the so-called Hodgkin's sarcoma is a reticulum cell sarcoma which shows more cellular pleomorphism than the typical reticulum cell sarcoma. However, as there is so much controversy as to whether the conditions generally diagnosed Hodgkin's disease are reactions to infection or are neoplasms, they are placed in a separate column but under the heading designating conditions of reticulum cell hyperplasia.)

I. Reactive Reticulocyte Hyperplasias

Examples are tuberculosis, leprosy, tularemia, and possibly the conditions of unknown etiology — Gaucher's and Niemann-Pick disease, which appear to be hyperplasias of reticulum cells.

II. Reticulocytoma, Leukemic — Syn. Monocytic Leukemia

The existence of this condition is now well established. Only one case is included in the Registry.

1. Leukemia of large cells of monocyte type having oval, bean-shaped or irregularly lobed nuclei and considerable basophilic pale cytoplasm. These cells are most difficult to separate from myelocytes, and differentiation by the blood film depends on precise staining. The cytoplasm is more abundant than in myelocytes, the size is more uniform and good staining brings out their essentially non-granular character. Accentuation of the acidophilic dye may show fine granules close to the nucleus. Too few cases have been observed to make further suggestions as to their differentiation, but the vital stains should be of value.

2. The spleen, lymph nodes and lymphatic structures are enlarged to varying degrees.

3. Microscopically there is a proliferation of rather typical reticulum cells between the nodules of lymph nodes and at the periphery of malpighian nodules in the spleen. The lymphatic nodules are not increased in size. Mitoses in the case observed were rare.)

4. The proliferation is characterized by a delicate meshwork of reticulum closely surrounding the individual cells.
5. Irradiation — no available data.

III. Reticulocytoma, Aleukemic — (Usually Termed Reticulum Cell Sarcoma)

1. The blood is aleukemic, though there may be an increase in monocytes, particularly after irradiation.

2. Enlarged lymph nodes without true metastasis.

3. Microscopically there is a diffuse hyperplasia of reticulum cells obliterating the node structure, with some areas and nodules of small lymphocytes remaining in the smaller and presumably more recently involved nodes.)

4. Reticulum surrounds each cell, forming a delicate meshwork.

5. Sensitivity to irradiation not determined by Registry material.)

IV. Reticulocytic (Reticulum Cell) Sarcoma

(1. Aleukemic: Monocytes may increase in the blood following irradiation.

2. Enlarged lymph nodes, spleen, or both, with metastatic deposits, invasion of adjacent tissues, or both.

3. Microscopically the cells are atypical reticulum cells surrounded by a delicate meshwork of reticulum. The cells show varying degrees of abnormality up to extremely bizarre forms in the most malignant types. Invasiveness may be slight, but infiltrative metastases involving parenchymatous organs, and especially skin, are frequent.

4. The intimate delicate reticulum constitutes a most important differential point for the diagnosis of this condition and its separation from large-celled lymphocytomas and small-celled carcinomas.

5. Sensitivity to irradiation not determined in Registry material.

HODGKIN'S DISEASE

(There are two distinct clinical forms of this condition, which to a large extent is localized, spreads by continuity and to local lymphatic structures, and sometimes terminates in sarcoma. The other is the

generalized form which spreads to nearly all lymph node groups, to liver, spleen or both, though there is usually a focus of greater intensity or degree of swelling.

I. Localized Form

1. Blood changes not diagnostic. In febrile phases there is a polymorphonuclear leukocytosis, and particularly in the later stages or after irradiation there may be an increase in monocytes. Eosinophiles are often moderately increased.

2. Local swelling of lymph node groups with more or less fusion of the nodes. Frequent sites are neck, mediastinum and abdomen. In the thorax the process may invade the lung by continuity or extend along bronchial lymphatics. In the abdomen the liver may contain nodules here and there without generalized involvement. The spleen may contain irregular masses. Small nodes are pale pink and translucent, but soon show areas of yellowish opacity and softening. Spontaneously, and especially after irradiation, there is much fibrosis which in large amounts is almost cartilaginous in consistence.

3. The earliest changes are seen in small nodes, that is, ones recently involved by the process, as the larger nodes show such complete obliteration of their structure that the sequence of events cannot be followed. In these small nodes there is a proliferation of the reticulum of the internodular stroma and more or less proliferation of the lymphocytes of the primary nodules. Large cells are formed and later the typical multinucleated Sternberg cells. The process is usually quite definite in the internodular stroma before the proliferating reticulum cells appear in numbers in the lymph nodules, also the new reticulum is quite dense between the nodules before it invades these structures. The reticulum is gradually replaced by collagen though the remnants of the lymph nodules can usually be made out. The lymphocytes gradually decrease during this sclerosing process, which goes on more rapidly if irradiation is used. Necrosis varies in different cases and appears to start at the node periphery in the early phases. No node of small size which could be considered the starting point of a Hodgkin's process has been described. It is possible that the primary lesion is characterized by early necrosis and that the proliferative changes are secondary. These localized processes in the gross cannot be distinguished readily from tuber-

culosis, especially the extrapulmonary lymph node involvements which are not rare in the negro race. Microscopically the absence of tubercle formation and the cell picture serve to differentiate. A considerable number of eosinophiles is usually seen in the tissue, especially in the early cellular phase.

4. The proliferating cells form reticulum, which is gradually replaced by collagen in the sclerotic process. In nodes almost wholly sclerotic small areas of reticulum representing former lymph nodules are surrounded by dense, relatively acellular collagenous rings representing the internodular stroma.

5. Irradiation increases the rapidity of the sclerosis and reduces the size of the swelling, and at the same time reduces the hyperplasia, especially that of the lymphocytes.

II. (*See Reticulocytoma, Leukemic*)

III. *The Generalized Form*

1. Blood changes not diagnostic. Febrile attacks, as in the localized form, may be accompanied by leukocytosis of the polymorphonuclear variety, while irradiation may lead to considerable increase in the monocytes, or these cells may increase spontaneously.

2. Starting usually from some one area of enlargement of lymph nodes or spleen the process causes in a relatively short time a generalized enlargement of lymph nodes and lymphatic structures. The generalized enlargement of the liver is especially noteworthy, though not always present to a marked degree. Grossly the organ is not characteristic but often the enlarged portal areas are visible. This type remains confined to the lymphatic structures, does not invade and clinically resembles the aleukemic lymphocytoma (pseudoleukemia).

3. Histological examination shows a cellular picture with less sclerosis, especially in the form that involves the lymphatic structures of the intestine. In the liver practically every portal space shows reticulum cell proliferation with enlargement while the Kupffer cells appear to be more abundant. In the spleen the proliferation is around the malpighian nodules and sometimes extends into them and obliterates the structure. The red pulp is not affected, except by enlargement of the nodules. In the nodes the process appears to start in the internodular stroma where there is proliferation of reticulum cells, which produce abundant reticulum of looser mesh than in the

localized type. There are fewer typical Sternberg cells but the large uninucleated reticulum cells are abundant, quickly invade the lymph nodules and increase the amount of reticulum. Sclerosis is slow and in the intestinal involvements little if any collagen is laid down. Necrosis is slight or absent. Eosinophiles are rare or absent.

4. This condition is characterized by the formation of abundant reticulum. Under irradiation there is sclerosis and collagen formation, but otherwise there is less sclerosis than in the localized form.

5. Sensitive to irradiation to some degree, but the generalization of the process renders adequate irradiation impossible.

IV. Hodgkin's Sarcoma

1. No cases with leukemia have been observed.

2. Occasionally during the course of a focal or generalized Hodgkin's disease there may occur a metastasizing neoplastic process, a true sarcoma. This sarcomatous change may come early or late in the course of the disease. It is prone to occur earlier in the more cellular types of lesion and at the younger ages. It may be the termination of slow processes, particularly those of a focal or localized nature which have been kept, sometimes years, under a certain degree of control by irradiation.

3. The cell of this tumor is the reticulum cell. The microscopic picture is that of reticulum cell sarcoma, sometimes with little pleomorphism, though usually there is much more than in the typical reticulum cell sarcoma. Metastases in other than lymphatic organs show less pleomorphism and fewer infiltrating lymphocytes, though, like true lympho-epithelioma, Hodgkin's sarcoma and reticulum cell sarcoma, they usually show considerable lymphocytic infiltration.

4. The reticulum is the same as is found in reticulum cell sarcoma.

5. There are no data available on the sensitivity of this condition to irradiation.

In the monocyte or reticulum cell group the characteristic differential criterion is the production of argentophile reticulum by the proliferating cells wherever these produce tissue or, in other words, are not purely infiltrative. In lymph nodes this is best seen in the internodular stroma; in the spleen in the peripheries of the malpighian nodules. In the sarcoma types the entire node may be involved, or only a part, as in a metastasis. Most of the reticulum cell

tumors composed of uniform, atypical cells are sarcomatous in that definite metastases occur.

Group III, the aleukemic reticulocytoma, is usually considered to be sarcoma, but there are a few cases in which the lesions were confined to lymph nodes and the changes were very like those of monocytic leukemia.

Reticulum cell sarcoma really includes Hodgkin's sarcoma and it is a better term for the condition.

The two types of Hodgkin's disease described appear to the writer as quite different conditions. The localized type has all the characteristics of an infectious granuloma, more like tuberculosis than any other known disease. The generalized type is more a hyperplasia with lesions closely resembling the cellular phases of the localized form, but without the tendency to necrosis and sclerosis.

The localized form frequently shows hepatic involvement in scattered nodules or massive infiltrations, just as is sometimes seen in tuberculosis. The generalized form, however, when it involves the liver affects *all* the portal areas, the lymphatic structures of the liver, in a manner similar to the lymphocytic involvement of these areas in lymphatic pseudoleukemia.

The material reviewed is still too scanty to justify the placing of this generalized form definitely as an aleukemic reticulocytoma, but it appears quite possible that such is the case and that it is not a reactive hyperplasia. Likewise many more completed cases must be accumulated to establish definitely that Hodgkin's disease in any form is an infectious granuloma. Termination in a definite sarcomatous condition is certainly unusual for granuloma but quite frequent in hyperplasias of lymphocytic and myelocytic tissue. The search for an etiological agent is definitely outside the scope of this Registry.

DISCUSSION

The foregoing statements are based on Registry cases in which adequate material and clinical data have been supplied. This classification is offered for criticism and to stimulate discussion and the contribution of cases which will either support, refute, or modify the opinions herein expressed.

✓ Considerable evidence has been presented heretofore that these conditions are closely related and that there have been cases showing

transitions between the groups arising from the different cells. This is especially true of the condition termed Hodgkin's disease. There is no evidence in the Registry material that this occurs. The term Hodgkin's disease, in recent years, has been applied to conditions of various types because of the presence of large multinucleated cells, or because of the clinical similarity between the cases, just as Thomas Hodgkin confused several of the groups over 100 years ago. We have ignored the facts that practically any malignant cell in some phase of its malignancy may produce multinucleated forms and that the clinical symptoms of a neoplastic condition are dependent on the location of the involvement, rather than on the type cell of the growth. In the Registry material myelocytomas, both granulocytic and erythrocytic, reticulum cell tumors, sarcomatous and not sarcomatous, and aleukemic lymphocytomas, especially the nodular type, have been diagnosed Hodgkin's disease. It is believed that error in the original diagnosis is thus responsible for the confusion that has led to the conception that Hodgkin's disease may terminate in either the lymphocytic or myelogenous group.

As Hodgkin's disease in its malignant phase is a true metastatic tumor, or as a true metastatic tumor may develop during the course of typical Hodgkin's disease, we must determine what the cell is that forms this malignant condition. In the Registry group the cells of the metastases in the sarcomatous phase of Hodgkin's disease have produced abundant reticulum and have not conformed to the morphology of either lymphocytes, large or small, or any cell of the myelogenous group. None of the other groups, lymphocytomas or myelocytomas, both of the granulocyte and erythrocyte types, produces reticulum. Monocytic leukemias and the reticulum cell tumors produce it in abundance. (In fact, many of the so-called Hodgkin's sarcomas cannot be differentiated from reticulum cell sarcoma. Hodgkin's sarcoma is often, but not always, more pleomorphic, while reticulum cell sarcoma sometimes shows considerable variation in the size and shape of the cells.

Skin lesions are occasionally found in all groups. Hemorrhagic types of lesion were present in all three cases of the erythrocyte group and are more frequent in the myelogenous than in the lymphogenous conditions.

The first phenomenon noted in reticulum cell involvements may be a skin manifestation, or the hyperplasia may appear to follow or

terminate a condition characterized by diffuse skin eruptions. Mycosis fungoides is allied to or is a type of the reactive Hodgkin's granuloma.

In general it has been found much more satisfactory to depend on the characteristics of the cell growth, its location and extension, than on the type of cell. Many of these cells cannot be distinguished from one another in the tissues, especially in the more or less atypical forms in which they occur in these tumor and tumor-like conditions.

It is fully realized that there are bizarre conditions arising from some of these cells that do now and will in the future defy classification. These often are seen in very early life and are rapidly fatal. Some may be embryonal tumors of a cell preceding any differentiation. Most of them are so malignant that death ensues before diagnosis or adequate study is made; however, most of this group of tumors and tumor-like conditions are proliferations of cells so far differentiated as to maintain certain diagnostic characteristics. Transformation of a cell that looks like a lymphocyte into a myelocyte, monocyte, and so on, may and probably does occur, but there is some doubt in such cases whether the cell was, in fact, a lymphocyte. Is it not more probable that it was a cell that could not be differentiated by the methods used?

It is of the utmost importance that adequate material, well prepared, be available for study, especially when one desires to classify the condition. A "chewed out" piece of lymph node or a poor section offers little possibility for diagnosis, except in the simplest cases. When lymph nodes are removed a large and small one both should be included. A well stained blood film is necessary.

SUMMARY

1. A discussion of the tumors and tumor-like conditions arising from the stem cells of the lymphocytes, the granular leukocyte, the red blood corpuscle, and the reticulum cell or monocyte is presented.
2. A classification of these conditions is presented, based on a study of the cases of the Lymphatic Tumor Registry of the American Association of Pathologists and Bacteriologists.
3. Certain criteria for the differentiation of the conditions are given in explanation or elaboration of the tabular presentation of the classification.

✓ 4. Certain evidence is presented that some conditions ordinarily classified as Hodgkin's disease belong to the reticulum cell group, either as reactive hyperplasias, aleukemic reticulocytomas, or reticulum cell sarcomas.

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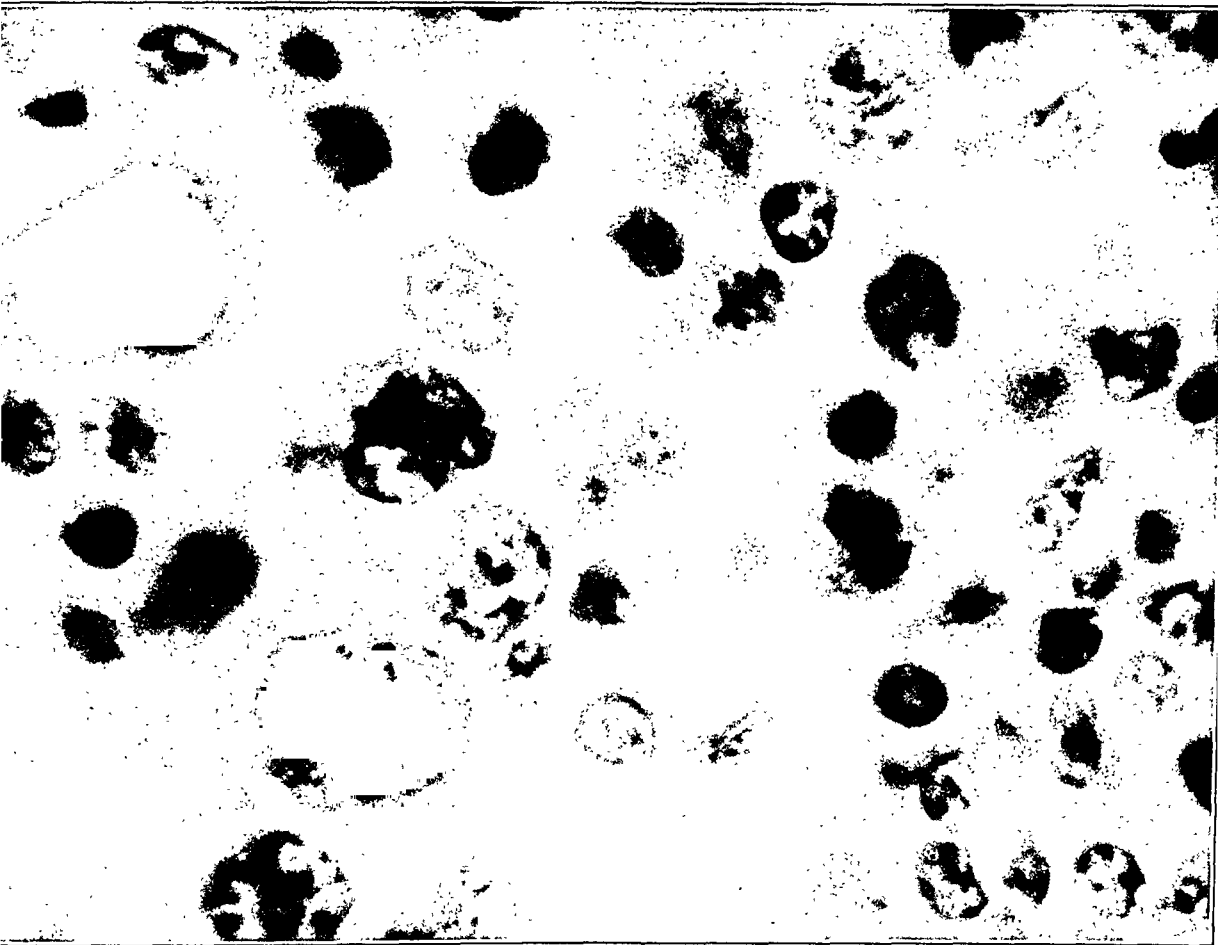
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DESCRIPTION OF PLATES

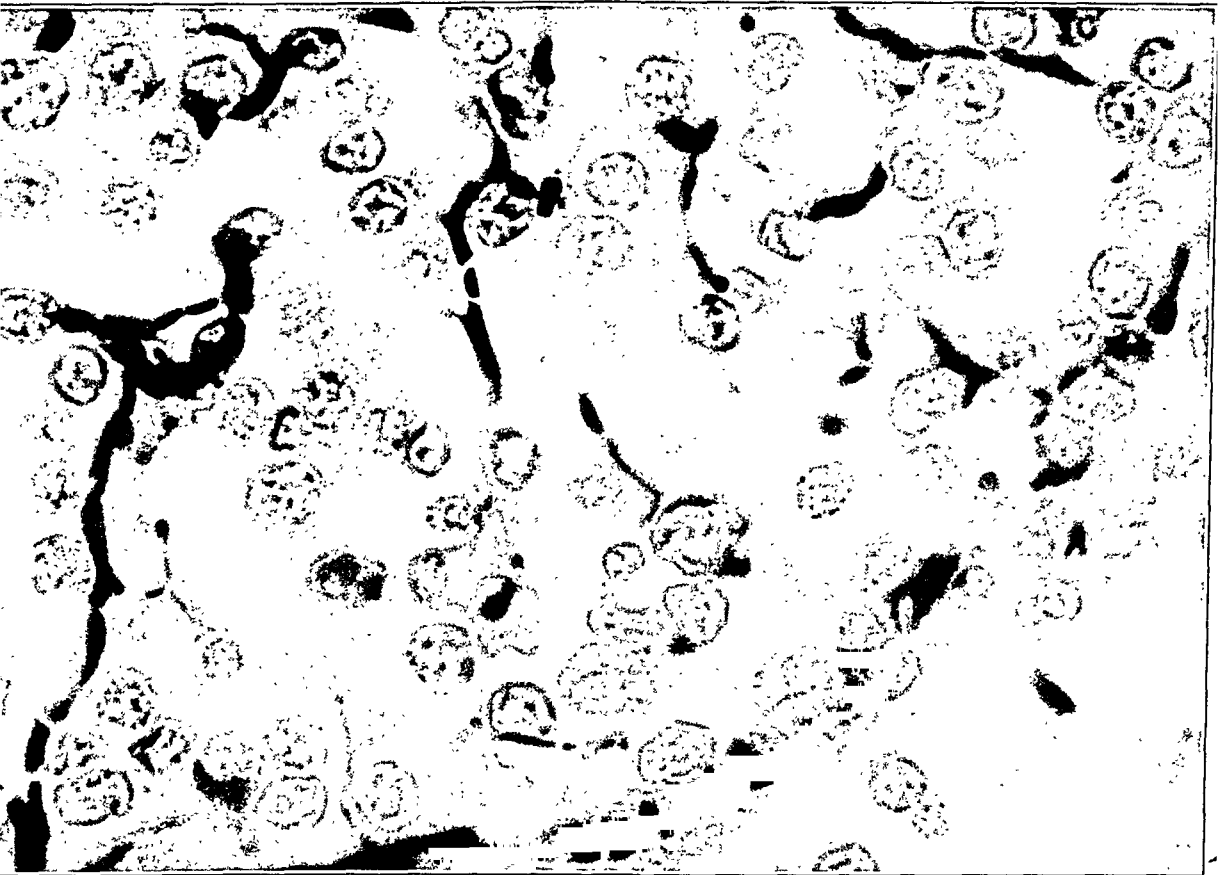
PLATE III

FIG. 1. Photograph of a section of lymph node from a case of lymphatic leukemia showing variations in cell size and giant cells. Hematoxylin and eosin stain.

FIG. 2. Reticulum stain from a section of the same node shown in Fig. 1. This is the characteristic reticulum of lymphocytic tissues. The lymphocytes do not form this reticulum. Similar reticulum is also seen in myelocytic hyperplasia. It is the normal reticulum of the node, produced by the reticulum cells. Compare with Figs. 6, 8, 10 and 12.



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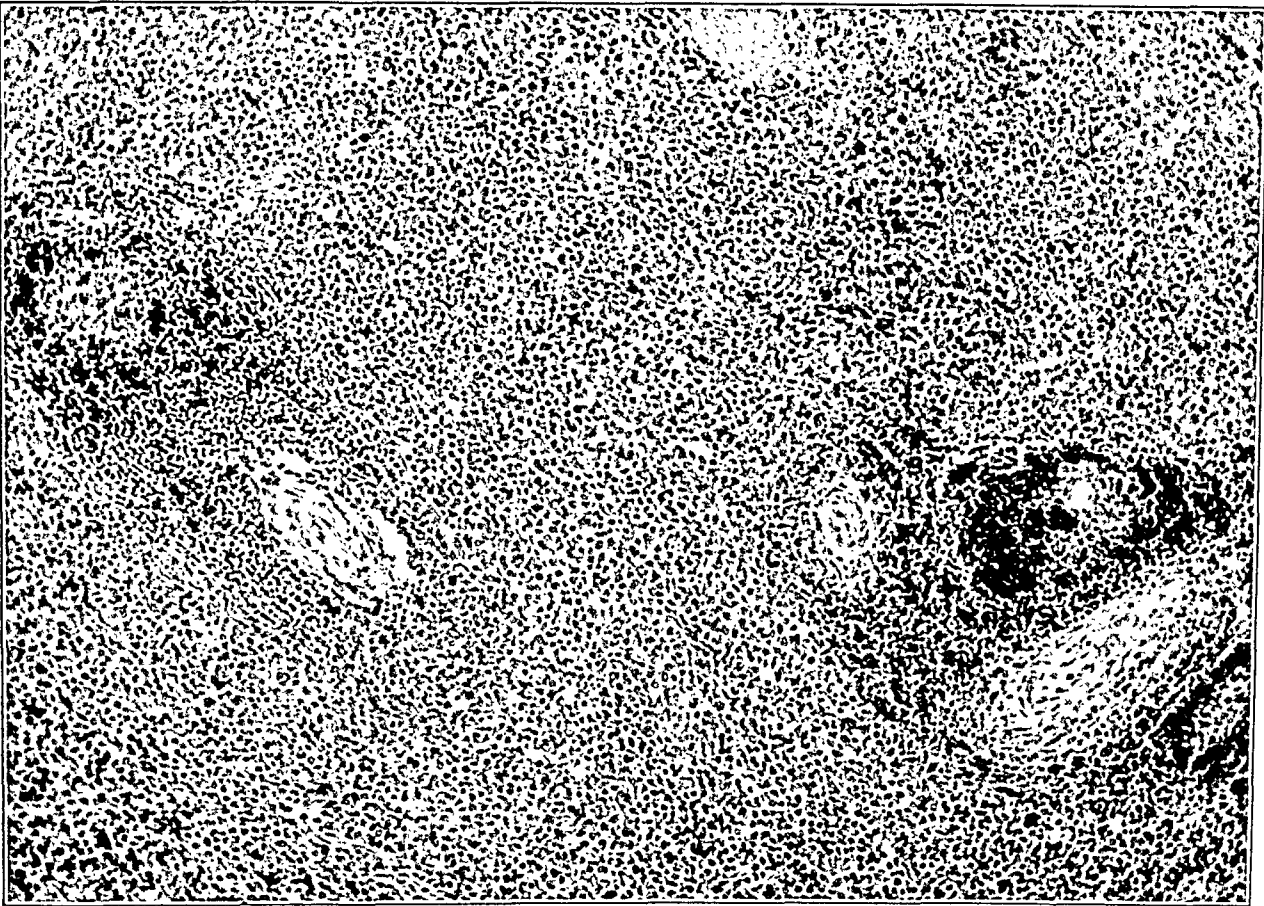


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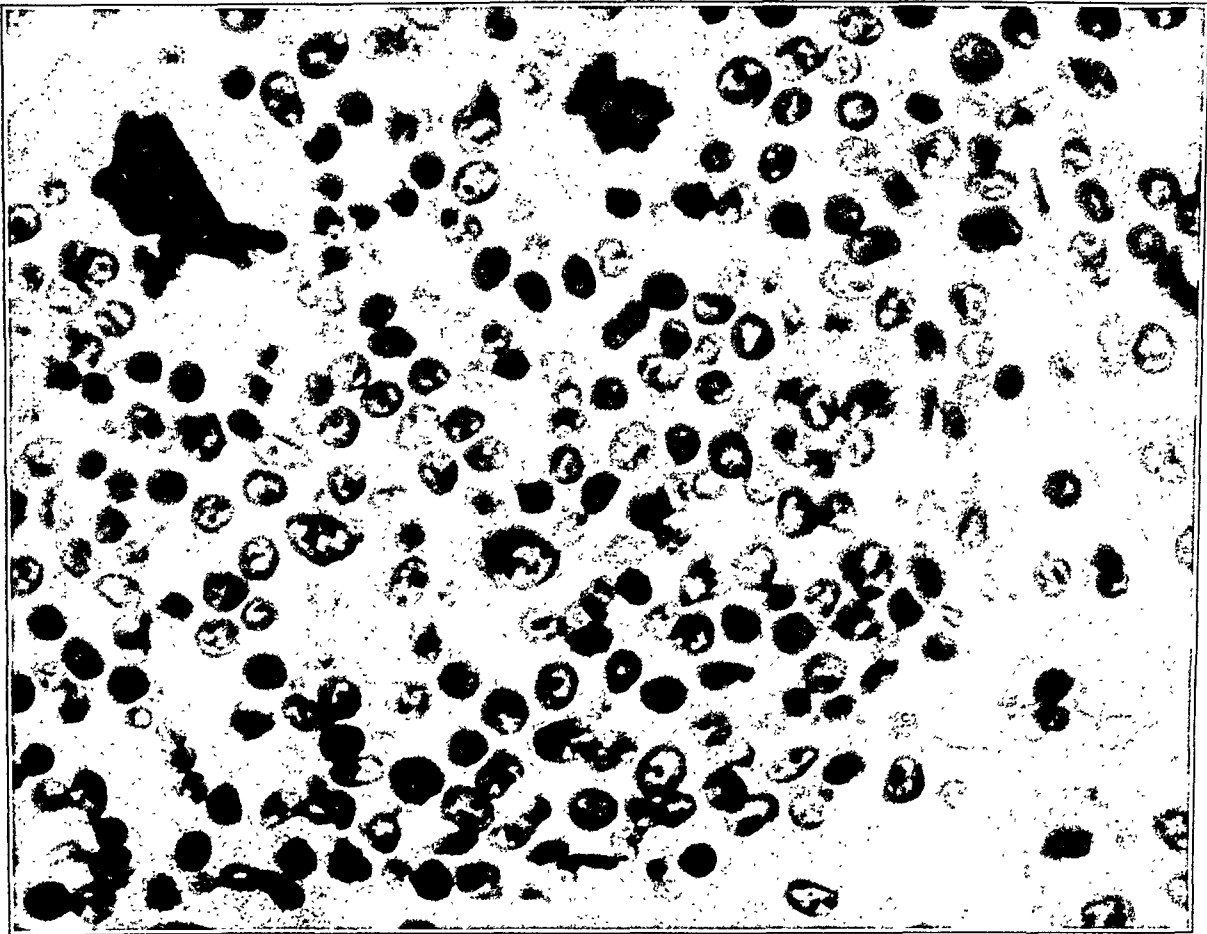
PLATE 112

FIG. 3. Spleen in myelogenous leukemia. Note the proliferation is between the nodules, which are somewhat atrophied. The change is in the red pulp, and differs distinctly from the change in lymphocytic proliferation in which the nodules are increased in size, encroaching on the red pulp.

FIG. 4. From a section of lymph node in erythrocytic leukemia to show typical megakaryocytes. Hematoxylin and eosin stain.



3

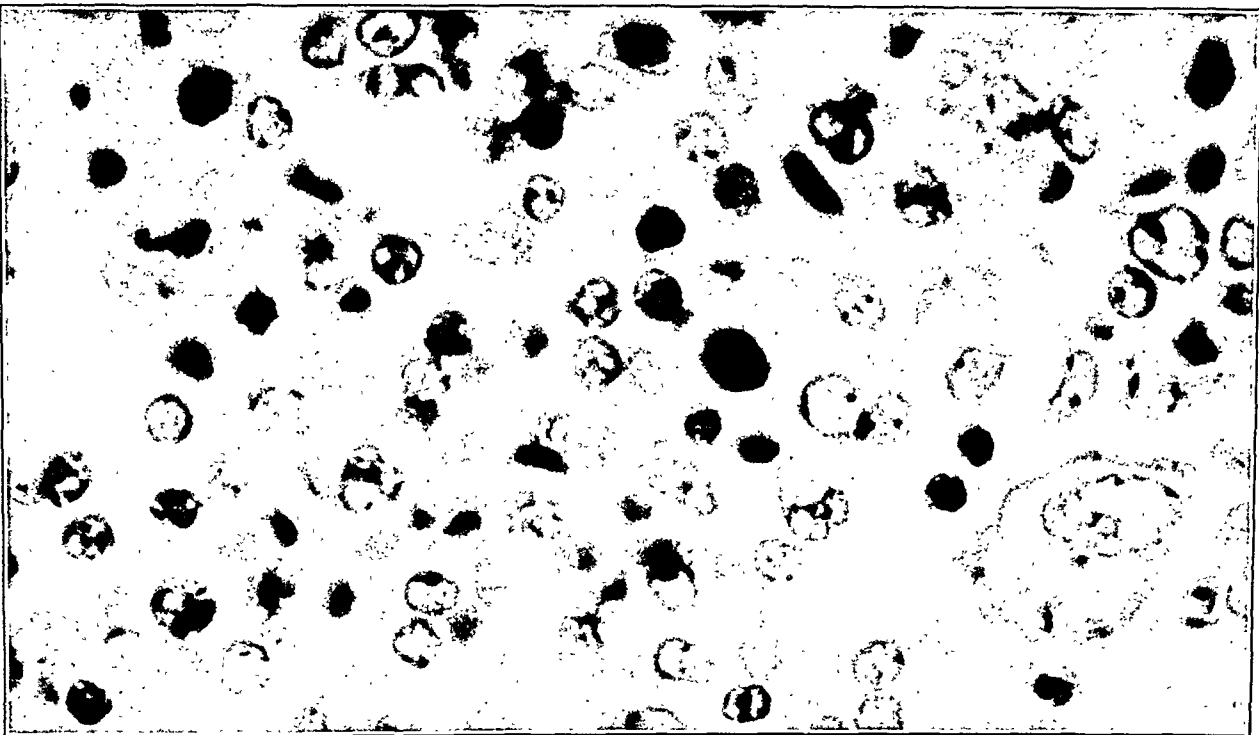


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PLATE 113

FIG. 5. Section from the same node as Fig. 4. A young megakaryocyte is seen in the lower right hand corner. The pyknotic cells are "blasts." Note a diffuse infiltration of red blood corpuscles, a picture suggesting that these have been formed locally.

FIG. 6. Lymph node in monocytic leukemia stained for reticulum. Note the characteristic reticulum in close contact with the proliferating cells. Note also that the proliferation is between nodules or around them. This nodule is decreased in size and is invaded to a slight extent at the periphery by the proliferating cells and the reticulum they produce.



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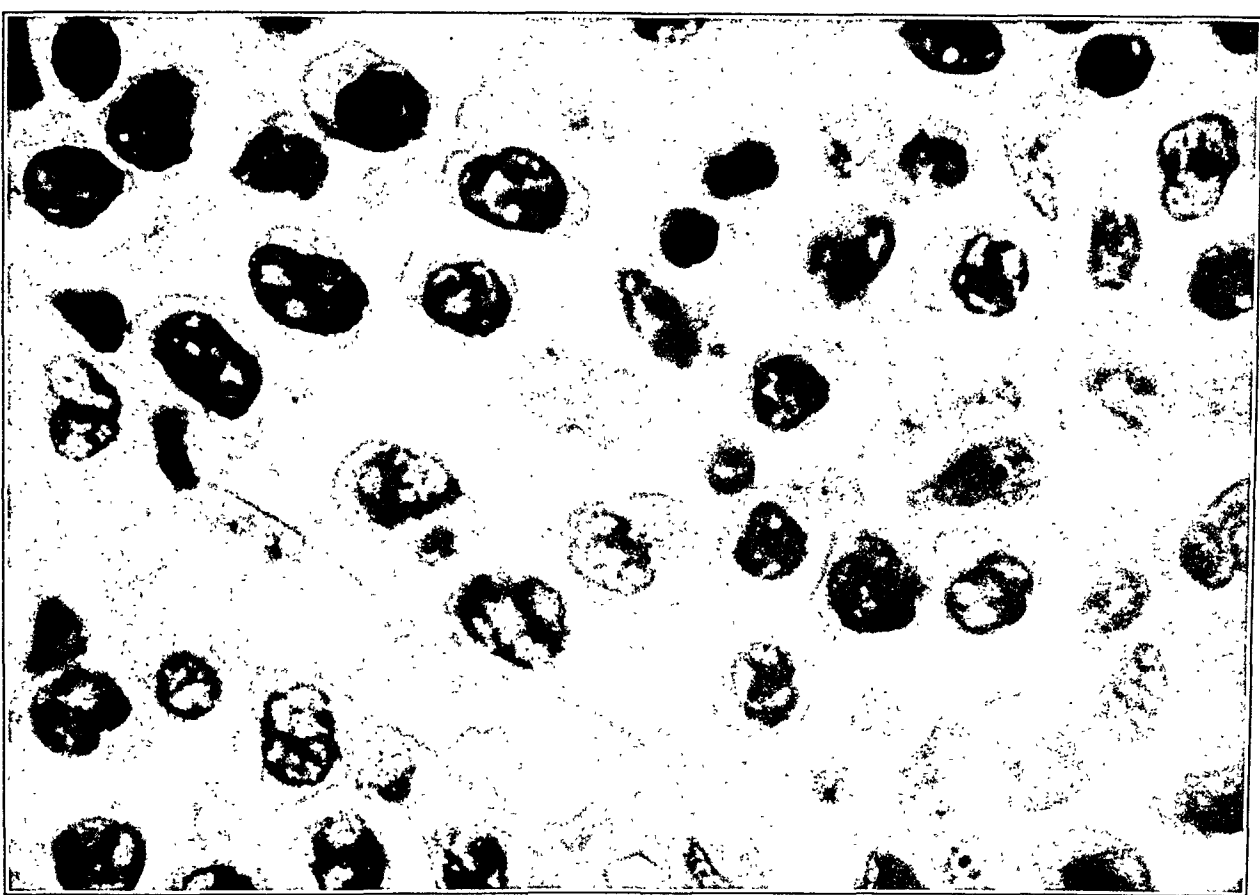


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PLATE 114

FIG. 7. Section of a lymph node from the same case shown in Fig. 6. Note the character of the cells. Without the help of differential staining of the intercellular reticulum it is difficult to differentiate such cells from large lymphocytes and marrow cells. Hematoxylin and eosin stain.

FIG. 8. Section from the same block as Fig. 7, stained for reticulum. Note the intimate relation of the reticulum to the proliferating cells. Figs. 6, 7 and 8 are from the same case.



7

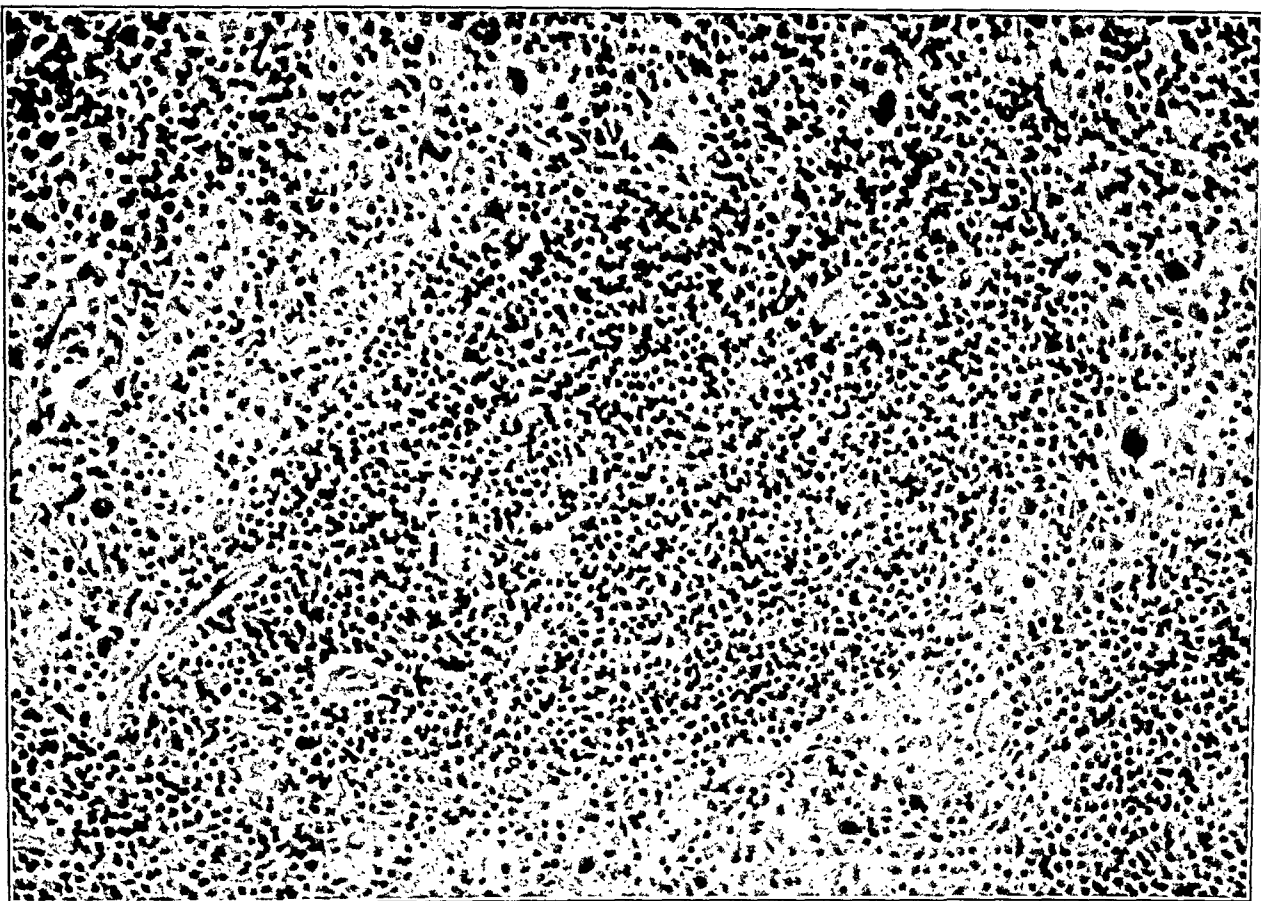


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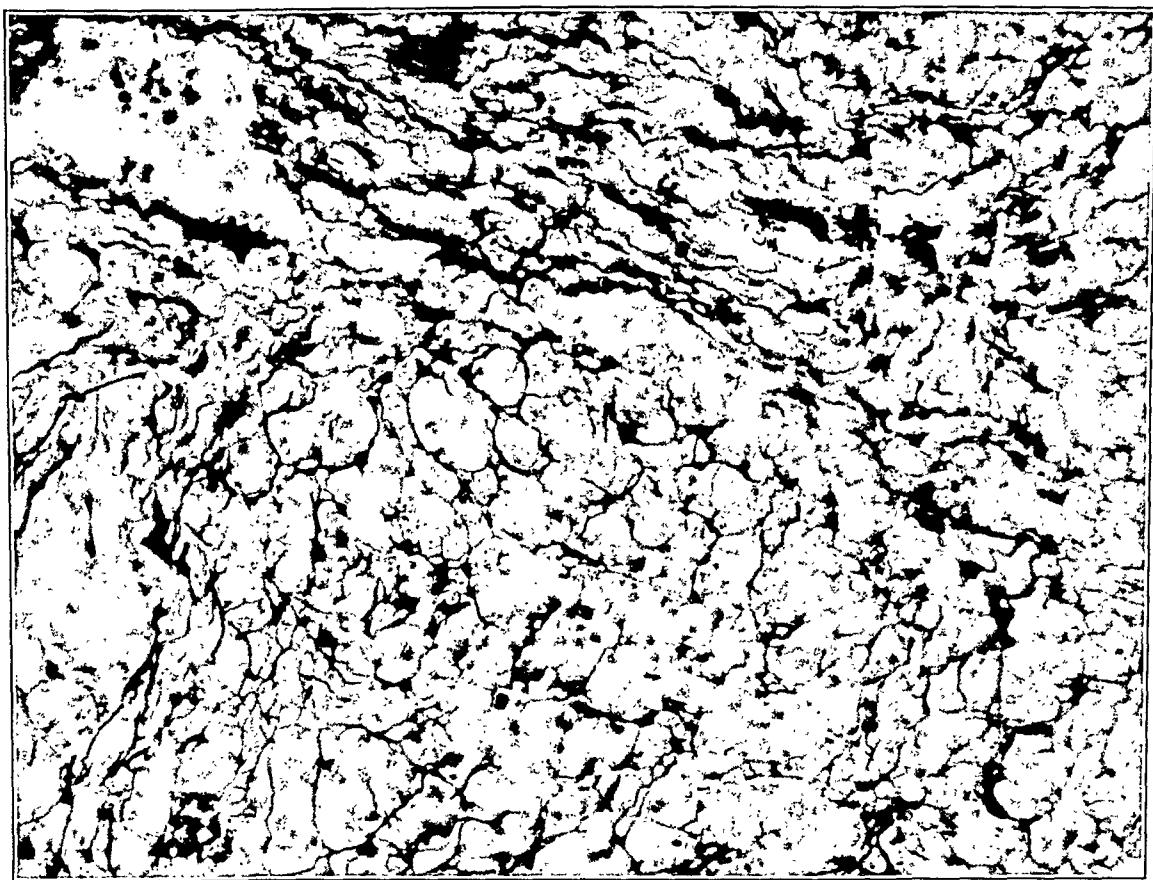
PLATE 115

FIG. 9. Spleen of Hodgkin's disease, reactive type. Note that the reticulum cell proliferation surrounds the malpighian nodule and has not involved the central part of the nodule. The nodules in this case were somewhat enlarged, as is frequently the picture at the beginning of the reaction. The decrease in nodule size appears to be due to the gradual encroachment of the process on the nodules, both in lymph nodes and in spleen, but the process starts as an extranodular reaction.

FIG. 10. Reticulum stain of a lymph node in the reactive type of Hodgkin's disease. Note the dense reticulum on the outside of the nodule shown in the upper and right hand portions of the photograph. The central and left hand portions are a part of a primary nodule, which at this time had become considerably infiltrated by the proliferating reticulum cells.



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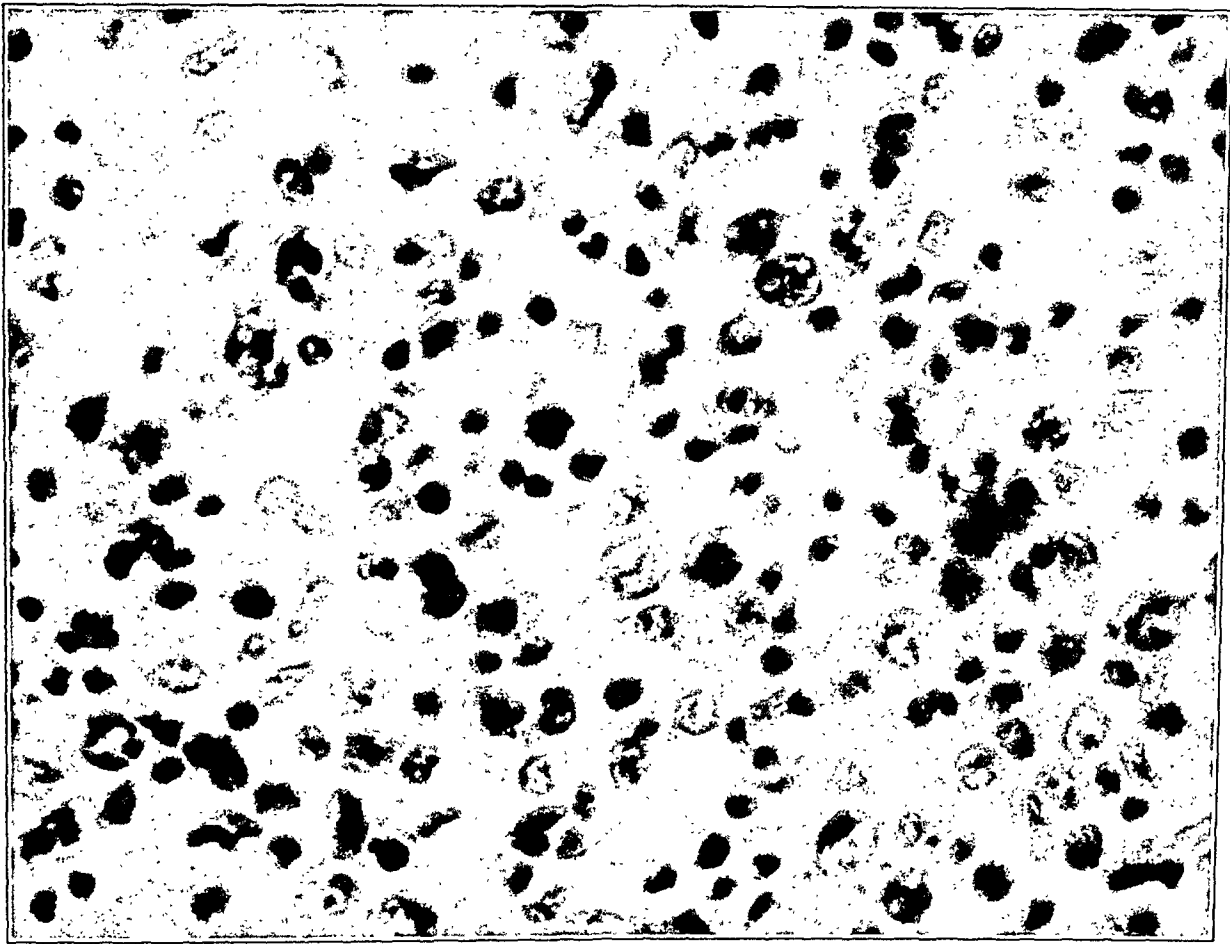


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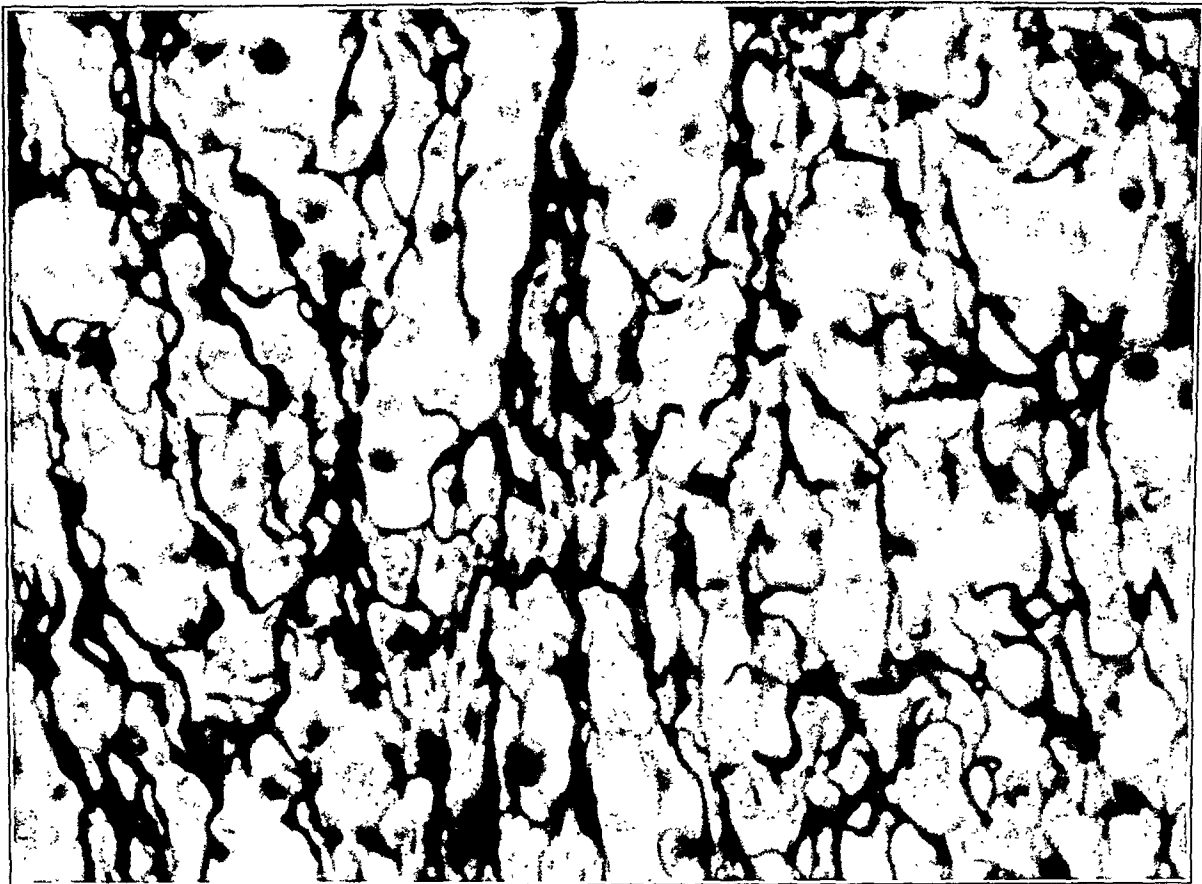
PLATE 116

FIG. 11. Section of a generalized Hodgkin's process. This is from a lymph node and shows the pleomorphic character of the process. Hematoxylin and eosin stain.

FIG. 12. Reticulum stain of a section from the same block as Fig. 11. This shows the characteristic reticulum of the diffuse Hodgkin's process and demonstrates the fact that it is a hyperplasia of reticulum cells.



11



12

STUDIES ON THE MYOCARDIAL ASCHOFF BODY *

I. DESCRIPTIVE CLASSIFICATION OF LESIONS

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Although Romberg¹ in 1894 is generally considered the first to have described the inflammatory lesions in the myocardium now known as Aschoff bodies, it would appear that they were really first reported by Goodhart² in 1879. This author observed interstitial cell growth around vessels and between myocardial fasciculi in a typical case of rheumatic fever that showed at autopsy verrucous endocarditis (hempseed size) of the mitral and aortic valves, and in all probability a fibrinous pericarditis. As is well known, the association between rheumatic fever and cardiac injury was suspected and noted long before this. However, this short introductory review will deal only with the historical development of our knowledge of the myocardial Aschoff body and will accordingly be limited to those workers whose contributions in this field marked a definite advance.

In 1887 Cadet de Gassicourt³ suggested that the inflammatory process in "rheumatism" starts in the depth of the muscular substance and is made evident by proliferation of interstitial tissue. In 1890 Krehl⁴ made the significant observation that in a case of acute verrucous endocarditis there were present in the heart perivascular infiltration, increase in connective tissue and changes in the coronary arterioles which were, in all probability, the lesions at present receiving considerable recognition. Krehl observed these processes occurring more frequently in the left ventricle, particularly under the auriculoventricular ring.

Romberg¹ also noticed the preponderance of infiltration at the tendinous valvular insertion line and in the inner and posterior portions of the left ventricle. His observation of increased interstitial tissue and the presence of large cells is significant, as are his descriptions of vascular involvement. Three months later Bret⁵ published

* Aided by a grant from the Lucius N. Littauer Foundation.

Received for publication April 26, 1934.

a more detailed description, referring to the cells as "embryonnaire," an observation which, in the light of modern conceptions on the origin of the Aschoff body, was quite remarkable. Bret observed enlarged pyknotic nuclei, a peculiarity in the staining properties of the cytoplasm, the occurrence of a caseous material surrounded by these large cells, all lying between the muscle fibers — in short, a picture that undoubtedly represents the type of Aschoff body that will be described in this report as the coronal variety.

The previously mentioned authors, however, failed to make what turned out to be the most critical and important contribution in the study of these lesions, namely, their recognition as specific for rheumatic heart involvement. It remained for Aschoff definitely to establish a new era in the study of rheumatic fever when he announced that these lesions were specific for this disease. In Aschoff's ⁶ 1904 report, based on a study of 5 cases of articular rheumatism from a collection of 150 hearts studied by Tawara, he observed the characteristic nodules in 2. Aschoff described the simultaneous occurrence of blood vessel lesions simulating periarteritis nodosa, the submiliary size of these nodules, the tendency for the cells to assume fan and rosette arrangements around central necrotic areas, the large indented nuclei, the presence of giant cells and the peripheral zone of polymorphonuclear leukocytes and lymphocytes. He suggested the ultimate transformation of the nodules into connective tissue, thus predicting future descriptions of the life cycle of the lesion, and believed the characteristic cells arose from leukocytoid elements derived from the adventitial cells of the blood vessels.

The following year Geipel ⁷ gave an excellent detailed description of his findings in the hearts from 5 cases of acute verrucous endocarditis of rheumatic origin. Believing the cells to arise from connective tissue this author was of the opinion that the "rheumatic poison" affects connective tissue with resulting cellular reaction and breaking down of collagen and cell cytoplasm. He observed that the nodules occurred in the 5th to 6th week after the onset of the disease, that they can reach a breadth of 80 microns and a length of 880 microns, can occur in the interstitial as well as perivascular connective tissue, that the giant cells result from both multiplication of nuclei and confluence of individual cells, and that the ground substance eventually becomes fibrillar and transforms itself into connective tissue — a description that remarkably portrays one aspect

of the life cycle of the lesion as we know it today. Because of the fact, however, that Geipel observed similar myocardial nodules in a case of renal arteriosclerosis without a history of rheumatic fever (but with adherent pericardium) he came to the conclusion that these lesions were not specific.

In 1906 Aschoff and Tawara⁸ reported 23 additional cases. In this paper the observation that the nodule did not bear as definite a relation to the blood vessels as had been stated before was recorded. They also added the description of the distinct nucleolus of the characteristic cell, mentioned the presence of mitotic figures and denied the origin of the giant cells from myocardium, mentioning the lymphocytoid cell as possibly giving rise to the characteristic cells. In the same year Aschoff⁹ discussed the appearance of nodules in the conduction system. In 1907 Coombs¹⁰ described the Aschoff body, confirmed its specificity and added the observation that the cytoplasm tended to be amphophilic. In 1908 Saigo¹¹ postulated an epithelioid and muscle cell origin of the Aschoff body. In 1909 Takayasu¹² described a variety of Aschoff body that will be reported in this paper as belonging to the mosaic type. This author did not consider the lesion strictly specific. In the same year Bracht and Wächter¹³ emphasized one of the most important histological properties of the cell cytoplasm, namely its basophilia, and also laid stress on the subendocardial sites of these lesions. In his 1911 report Coombs¹⁴ stated that the ground work of the Aschoff body may contain fibrin, that the auricles are rarely the site of these lesions and that the papillary muscles and septum usually escape the rheumatic damage, whereas the central fibrous body and the tissue around it are especially susceptible to the inflammation. In 1914 Thalheimer and Rothschild¹⁵ again emphasized the specificity of the Aschoff body, mentioned the presence of several types of lesions in the myocardium, and described the "streamer-like" processes of the cytoplasm. In the same year Mallory¹⁶ described the degenerated collagen fibers slowly attacked by endothelial leukocytes which sometimes formed giant cells.

In a discussion of a paper by Huzella¹⁷ before the German Pathological Society in 1914 both Fraenkel and Aschoff again drew attention to the chief rôle played by connective tissue injury in the development of these lesions. In 1921 Fahr¹⁸ compared the tissue changes in the myocardium and knee joints as they occur in scarlet

fever and in acute rheumatic fever. While pointing out certain similarities in the myocardial lesions he definitely considered that the qualitative and quantitative differences in the two lesions were sufficient to establish the rheumatic lesion as specific. In his 1924 monograph Coombs¹⁹ expressed the belief that the characteristic cell in the Aschoff body developed from vascular endothelium.

In 1926 Sacks²⁰ published his excellent review on the pathology of rheumatic fever in which he suggested the subcutaneous nodule as a fruitful source for studying the origin of these cells by means of supravital stain technique. Sacks considered these cells as possibly arising from histiocytes. In 1929 Talalajew²¹ made an important contribution to the study of Aschoff bodies, emphasizing again the important rôle of collagen swelling and necrosis and describing three phases in the life cycle of the lesion, namely swelling of collagenous bundles with exudation, proliferative processes with development of syncytial masses, and finally sclerosis. In this paper Talalajew attempted to correlate the evolution of the lesion with a time component. He believed that the first phase (exudative) may be seen at the end of the 2nd or 3rd week of the disease; the second phase (proliferative) may be completely developed at the end of the 1st or 2nd month and last, at times, for 6 months. The third phase (sclerosis) may appear during the 2nd month and may last for 6 months. In the same year Clawson's²² observations led him to doubt the specificity of these lesions. He did not believe that they have distinctive histological characteristics and claimed that they are at times found in other diseases (subacute bacterial endocarditis, scarlet fever and syphilis). He observed all stages in the histological appearance of these lesions between polyblastic types and abscess-like forms. In the same year also, Gross, Loewe and Eliasoph²³ described unsuccessful attempts to transmit the disease to animals, laid down certain criteria whose fulfilment was essential in order to justify the claim, on a histological-anatomical basis, that the disease has been experimentally reproduced and, in a discussion on reported work, concluded that this disease as yet had not been successfully transmitted to animals.

In a report on the results of our studies on Aschoff bodies, presented before the American Association of Pathologists and Bacteriologists in 1930, we²⁴ described several types of Aschoff bodies probably representing different stages in the life cycle of this lesion.

We also described the development of a network of argentophilic reticulum fibers and emphasized the collagen swelling as the conspicuous feature of the early injury. Shortly after this Klinge²⁵ and his co-workers published a series of reports dealing with the pathogenesis of rheumatic fever in which, amongst other observations, they stressed fibrinoid degeneration of the ground substance as being the conspicuous feature of the early lesion (*Frühinfiltrat*). They also observed the characteristic silver-staining reticulum and described the Aschoff body in subacute and chronic stages, the healing scars and the lesions in the recurrent cases. They were strongly impressed by the allergic and hyperergic (Swift, Derick and Hitchcock²⁶) concept of the origin of these lesions and believed that they had reproduced Aschoff bodies by properly sensitizing animals. They considered the characteristic cells to arise from the mesenchymal elements, a conception all the more interesting in view of Bret's hypothesis 35 years before this work appeared.

In 1930, however, Fahr²⁷ again emphasized the histological differences between the lesions found in scarlet fever and in rheumatic fever and denied the identity of Klinge's experimental lesions with those of true rheumatic fever. In 1932 McEwen²⁸ made supravital stains on scrapings from subcutaneous nodules. He concluded that the essential elements are cells arising from the mesenchymal elements of loose connective tissue, although endothelial cells may take part in this formation. These cells are characterized by their almost complete lack of phagocytic power and are not characterized by the reactions with neutral red which distinguish monocytes, epithelioid cells and clasmatoocytes.

In his monograph on rheumatism published in 1933 Klinge²⁵ compiled the numerous reports published by himself and his co-workers and extended some of the observations. With regard to the life cycle of the Aschoff body he found that at the end of the 2nd week of the illness this lesion is represented by swelling of the "ground substance" of the collagen bundles, increase in connective tissue and wandering cells, with the presence of occasional giant cells. After the 4th week many swollen and multinucleated cells are found arranged either as a rosette around the swollen collagen, or dispersed throughout it. The center of the lesion gives a fibrin stain. The cells may show cytoplasmic streamers and present poorly outlined edges. An argentophilic reticulum is present. From this point

on, involutionary changes take place through the disappearance of the giant cells and fibrin and the development of connective tissue cells. In the last stages loss of the argentophilic properties takes place and the mass is converted into scar tissue.

It is obvious from this brief review that considerable obscurity still exists on the nature, histology and specificity of the Aschoff body. At least one of these points appears to be amenable to fairly exact study, namely, the histology of the lesion. It seems reasonable to assume that much of the confusion in the descriptive literature is due to the fact that different stages in the life cycle of the lesion have been emphasized by various authors as the typical Aschoff body. In this report we propose to present a simple histological classification and description of the lesions based on a study of 70 hearts obtained from cases in which diseases other than rheumatic fever could reasonably be ruled out. It will be seen that the lesions fall naturally into a few definite and more or less easily recognizable categories which, parallel clinical studies lead us to believe, reflect at once the stages in the life cycle of the lesion, as well as individual reactions of the given tissue.

So many authors have emphasized the dominant rôle played by injury to the collagen framework in the development of the Aschoff body that this can be considered an accepted fact. While a considerable concentration of collagen is found around blood vessels and in the subendocardium its distribution in the heart, considered as a whole, is not so widespread around the vascular bed and subendocardium as it is in the interstitial tissue between the muscle bundles. It is not surprising, therefore, that while the perivascular and subendocardial situations of the Aschoff body have been repeatedly emphasized in a considerable number of reports, many authors have observed that this lesion is by no means confined to these sites. On the contrary, even though they appear to be the sites of predilection in a number of cases, it would seem that the interstitial myocardial connective tissue is much more frequently involved. It is in this looser tissue that the lesions present a series of evolutionary phases relatively unhampered by dense collagen and elastic tissue and where, therefore, their life cycles can be traced in their more striking and characteristic forms. It is for these reasons that our descriptions and classification will be confined to the Aschoff body found in the interstices of the myocardial bundles. These will be referred to as

"myocardial Aschoff bodies," in contradistinction to the lesions found in the endocardium, subendocardium, adventitia of the blood vessels and in other organs. In the case of Aschoff bodies occurring in other organs the difficulty of obtaining sufficient material for statistical studies renders it inadvisable to attempt a classification at this time. In some tissue, *viz.*, subcutaneous nodules, tendon sheaths, and so on, legitimate doubts exist concerning the identity of these lesions with those found in the interstitial tissue of the myocardium. The nodules found in the adventitia of the blood vessels, endocardium, subendocardium and pericardium will be discussed briefly.

GENERAL CONSIDERATIONS

It has been mentioned already that the origin of the characteristic cell of the Aschoff body has been variously attributed to "leukocytoïd elements" from adventitial cells, myocardial cells, epithelioid cells, endothelial leukocytes, vascular endothelium, histiocytes, "polyblasts," "lymphocytoïd cells," connective tissue cells and undifferentiated mesenchymal elements. A careful study of considerable material indicates that only the three latter sources deserve serious consideration. While a study of subcutaneous nodules, as carried out by McEwen,²⁸ is of considerable importance, one cannot make the basic assumption that one is here dealing with a process identical with that occurring in the Aschoff body, even though the etiological agent is the same. Certainly, the subcutaneous nodule presents a histological appearance that is different from that of the Aschoff body, and apparently lacks the clear-cut specificity of the latter (Saphir and Wile²⁹). It is important in this connection that of the eight authors cited by McEwen as discussing the origin of the characteristic cells in the Aschoff body, and in the subcutaneous nodules, seven suggest a different origin for each.

Studies on the Aschoff body very early in its development invariably disclose a swelling of the interstitial collagen fibers as the most conspicuous phenomenon. The lesion shows swelling and fusion of the fibers with development of intense eosinophilic properties. According to Klinge,²⁵ these fibers do not undergo dissolution. The ground substance swells, takes the picric acid stain with Van Gieson's method and stains with fibrin methods. On the other hand, argentophilic fibrils are preserved. With the resolution of the in-

flammatory process these newly acquired tinctorial properties disappear.

Even in these very early stages two distinct types of lesions are recognizable. One type, apparently arising in those areas where the collagen occurs in the form of relatively large compact masses, maintains for some time this dense collagenic structure and eventually gives rise to what we shall term the coronal Aschoff body (Fig. 1). The other type arises in the looser connective tissue where the collagen strands occur as more or less isolated fibers. This eventually gives rise to the reticular type of Aschoff body or "*Frühinfiltrat*" of Klinge (Fig. 2).

Simultaneously with the swelling of the collagen one observes a local accumulation of small round cells with spherical nuclei and extremely inconspicuous cytoplasm, indistinguishable from lymphocytes (Fig. 3). Whether these cells actually represent lymphocytes or originate from previously existing fibroblasts, or from histiocytes, *i. e.*, mature differentiated cells, or from the undifferentiated mesenchyme (Hueck³⁰), it is as yet impossible to determine. It seems best, therefore, to designate these original cells as "mesenchymal cells" in order to avoid further controversy, bearing in mind the fact that this designation does not necessarily connote a direct derivation from the primitive mesenchymal system that has not passed through a differentiated cell stage (lymphocytes, fibroblasts, histiocytes). Already in this early stage Pap's³¹ stain discloses the development of argyrophilic fibrils in close proximity to the proliferating mesenchymal cells, as well as along the swollen collagen fibers (in the reticular form).

Up to this point the lesion cannot safely be considered specific for rheumatic fever. Inflammatory lesions of the myocardium occurring in other diseases have also been seen to present interstitial collagen swelling with local cellular proliferation. In the case of the Aschoff body, however, additional phenomena appear from this point on; which, taken as a whole, present a picture that we have never seen in any disease where rheumatic fever may safely be ruled out. We must, therefore, conclude that they are absolutely specific for this disease.

With the introduction of the additional histological features (*vide infra*) which give the Aschoff body its strictly specific characteristics, development may take place along several different lines. The

histological structure of the lesion apparently depends on whether the Aschoff body originates from the dense or the loose collagen, on that phase of the life cycle of the lesion that is being studied, and also to some extent on the individual reaction occurring in the given case. A study of considerable material makes it clear that there is a consistency in the characteristics that these developing myocardial Aschoff bodies assume and, on the basis of the topographical relations of the cell and tissue structure, as well as on their tinctorial properties, the lesions may be placed into one of the following categories.

CLASSIFICATION OF MYOCARDIAL ASCHOFF BODIES

1. Small cell coronal type
2. Large cell coronal type
3. Syncytial coronal type
4. Reticular type
5. Mosaic type
6. Polarized type
7. Fibrillar type

In the description of these types of Aschoff bodies mention will be made of their form. The question that naturally arises is whether this may not be influenced by the plane through which a given section is cut. It may be stated at once that our description is based on a reconstruction of the shape of the Aschoff body gathered from observations of the lesions, as observed in a great many sections and, in some cases, on the study of serial sections. From these studies it appears that while the nodules are at times spherical they generally take an oval, disk-like or spindle form. During the later phases of the life cycle of the lesion the disk-like and spindle forms predominate, finally elongating themselves into fusiform scars that lie in the interstices of the myocardial bundles.

Before entering into a detailed description of the various types of myocardial Aschoff bodies listed in our classification brief mention should be made of the histological characteristics of the Aschoff bodies situated in the adventitia of blood vessels and in the endocardium, subendocardium and pericardium. The topography of these lesions as a whole, as well as the relation of the cells to the collagen framework, is apparently determined by the density and

configuration of the tissue in which they lie (Gräff,³² Darré and Albot³³). Thus, the endocardial and subendocardial Aschoff bodies are represented by compressed, somewhat elongated lesions which tend to assume the form that will be called in this report the mosaic variety. The cells are scattered throughout the swollen collagen framework. If the collagen bundles are dense and compact the cells are irregularly compressed, assume bizarre polymorphous shapes, and may be connected to one another by delicate cytoplasmic streamers. As these Aschoff bodies pass through their life cycle, the cells change their shape and structure in accordance with the description that will be given for the myocardial lesions. The structure of the lesion as a whole, however, still remains that of cells compressed by a dense connective tissue.

In the case of pericardial Aschoff bodies the very loose milieu and the scarcity of collagenous bundles produce, during the early stages, a diffuse structure somewhat resembling the reticular type (*vide infra*). The development of the silver-staining lattice is not interfered with and the lesions show the tinctorial changes present in the myocardial nodules, the difference again being largely determined by the topographical relations of the cells to the surrounding tissue. The perivascular Aschoff bodies, on the other hand, generally occur in one of three forms, again determined by the consistence of the adventitial fibro-elastic tissue. A frequent form is the compressed mosaic type such as was mentioned for the endocardial and subendocardial Aschoff bodies. Here the Aschoff body appears to wrap itself around the vessel in the form of intercryptic cells compressed by dense connective tissue. Another form is in a looser mosaic pattern in which the cells predominate over the collagen framework. They are often large and appear frequently in giant cell form with the cells in fairly close juxtaposition. Finally, the parallel arrangement of the collagen fibrils along the course of a vessel not infrequently produces a picture that resembles the polarized and fibrillar types to be described. It should be mentioned again, however, that in spite of the configuration thus impressed on these Aschoff bodies by the state of the perivascular fibro-elastic tissue, the cells themselves undergo an orderly progression of changes similar to those that occur in the myocardial Aschoff bodies proper.

Finally, concerning the properties of all types of Aschoff bodies found in the heart, it may be said that, contrary to what has been

stated in the literature, these lesions rarely, if ever, show a fibrin constituent. Furthermore, the presence of the Aschoff body does not influence any elastic tissue present at the site of the lesion. Particularly during the stage of fragmentation portions of the swollen collagen may take the picric acid stain with the Van Gieson method. All stages of Aschoff bodies may show a mantle of polymorphonuclear leukocytes (neutrophilic and eosinophilic), lymphocytes, plasma cells and fibroblasts. These non-specific constituents of the lesion are generally found around the young fresh nodules so frequently seen in the myocardium of children. On the other hand, a variety of lesions which present no specific characteristics may also be found. These may vary from interstitial edema with relatively few wandering cells, to large collections of leukocytes, particularly eosinophiles (Wätjen ³⁴), even to abscess-like formation.

Mitotic figures occur with extreme rarity. On the other hand, dissolution of the Aschoff cells is not infrequently encountered. This is usually preceded by disappearance of nuclear chromatin, leaving nuclear ghosts in the form of more or less empty vesicles. Damage to the myocardium adjacent to the Aschoff bodies may be relatively mild. At times, however, considerable destruction takes place. The neighboring myocardial cells may appear hypertrophied, at times vacuolated, and may show complete dissolution and replacement by enormous scars. As a consequence, whereas the Aschoff body is essentially submiliary and visible only microscopically, the extensive scarring of the adjoining muscle, as well as fusion of neighboring Aschoff bodies, may produce lesions that are macroscopically visible (MacCallum ³⁵). It may be added that eosinophilic masses may be seen within the cytoplasm on rare occasions. These were considered by Coburn ³⁶ to be engulfed collagen particles. Bacterial stains almost invariably show the lesions to be free of bacteria. The organisms that may rarely be seen can easily be accounted for as of postmortem occurrence.

METHODS

The hearts were generally fixed in 10 per cent neutral formol-saline.* At times formol-Müller was used as a fixative. Before fixation blocks of tissue were removed and placed in 96 per cent alcohol and in Zenker's solution for special

* Solution of formaldehyde U.S.P. 10 parts, 1 per cent sodium chloride solution 90 parts. This solution is rendered neutral with a weak alkali.

staining purposes. For routine studies the standardized blocks of Gross, Antopol and Sacks³⁷ were cut after fixation and sections were stained with hematoxylin and eosin, and with Weigert's elastic and Van Gieson's connective tissue stains. In order to study the development of argentophilic fibers Pap's silver impregnation method was used. There were employed further, Masson's trichrome stains, Goodpasture's, and Brown and Brenner's bacterial stains, Mallory's phosphotungstic acid hematoxylin and Weigert's fibrin method. We have abandoned the Unna-Pappenheim methyl green-pyronin method inasmuch as in our hands this procedure has not given results that were in any sense superior to or more edifying than those obtained with a properly performed hematoxylin-eosin stain. In carrying out the latter attention should be paid to differentiating the hematoxylin-stained tissue in weak alkaline water. By this means the basophilic properties of the cytoplasm are brought out clearly, without losing the other important characteristics of the cells, namely the protoplasmic structure and the ragged edges.

SMALL CELL CORONAL TYPE

This type of Aschoff body (Fig. 1) is generally somewhat ovoid in shape. Occasionally, round as well as more elongated forms are also encountered. Early in its development one may note in many examples of this type of Aschoff body a considerable accumulation of rather small cells (slightly larger than a lymphocyte) which form a generally compact mantle of varying thickness around a central mass of swollen eosinophilic collagen. Because of this peripheral situation of the cells with respect to the central collagen mass this type of Aschoff body will be referred to as the coronal type.

The collagen is, as noted above, swollen and eosinophilic. In most instances the fibers are fused into large irregular masses. The swollen collagen may be seen to be partially or completely broken up into fine or larger granules (collagen fragmentation). The cells are round or oval, the cytoplasm stains evenly, is generally basophilic and forms a delicate and distinct, smoothly outlined mantle around the nucleus. At times the cell may possess more than one nucleus (two or three centrally placed). These giant cells, however, occur less frequently in the small cell coronal type of Aschoff body than in any of the other types to be described, except possibly the fibrillar type.

The nuclei may occur in three forms commonly found in all varieties of Aschoff bodies. The type of nucleus found most frequently in the small cell coronal Aschoff body is round or oval with a delicate, sometimes folded nuclear membrane and a fine dust-like chromatin structure, which may at times show irregular concentrations or bar-

like arrangements with fine projections radiating from the bar. Because of its resemblance to the fibrocyte (fibroblast) this nucleus will be referred to as the fibrocytoid nucleus (Figs. 1, 4, 9, 10, 11). The next most frequently occurring variety is the owl-eyed nucleus (Figs. 4, 5, 6, 9, 11, 12). This has been termed "target" nucleus by Whitman and Eastlake.³⁸ It is generally somewhat irregularly circular, possesses a heavy nuclear membrane with a distinctly dark and, at times, somewhat stellate nucleolus. The space between the nucleolus and the nuclear membrane tends to be poor in chromatin material. This is well brought out by the Masson stains. In a considerably smaller percentage of the cells the nucleus is somewhat polymorphous in shape and generally quite large. It stains solidly and is therefore properly designated pyknotic nucleus (Figs. 11, 12, 15, 19).

The cells in this type of Aschoff body may be so numerous, and the collagen at times so scanty, that the impression given is that of a rather compact cellular subvariety. This type of Aschoff body not infrequently shows a fairly conspicuous mantle of leukocytes among the other layers of the small cells, spreading diffusely into the adjacent myocardium.

LARGE CELL CORONAL TYPE

This type of Aschoff body (Figs. 4 and 5) is somewhat similar morphologically to the small cell variety. As its name indicates, the central swollen and at times fragmented mass of eosinophilic collagen is surrounded by a mantle of cells which possess much more abundant basophilic cytoplasm. The cytoplasm may be finely granular and not infrequently presents ragged edges, that is to say, the cellular outline tends to be indistinct, drawn out into pseudopods and broken up into granules. In this type the incidence of giant cells is larger than in the small cell coronal variety. As in most types of Aschoff bodies the giant cells are characterized by a central position of the nuclei. In the large cell coronal Aschoff body the giant cells are larger and may possess seven or more nuclei; the latter are round or oval and are irregularly arranged at the center of the cell. Silver stain reveals a definite reticulum in the proximity of these cells.

SYNCYTIAL CORONAL TYPE

This type of Aschoff body (Fig. 6) occurs relatively infrequently and has been found thus far generally in acute cases. The conspicuous feature of this lesion is the development of an enormous syncytial mass, or masses, which apparently overshadow the scant central collagen material. The cytoplasm is extraordinarily abundant, basophilic, with indistinct and ragged outlines. Isolated fragments of cytoplasm are found. The nuclei are predominantly owl-eyed and appear to take a peripheral position within the cytoplasmic masses. Fibrocytoid nuclei also occur. Pyknotic nuclei are relatively infrequent.

RETICULAR TYPE

It has been mentioned already that the reticular type of Aschoff body arises in loose connective tissue. The framework consists of an interlacing mesh of swollen, eosinophilic collagen fibers showing fusion at points of contact (Fig. 2). In its more characteristic form the meshwork shows an orientation directed more or less along the planes of the adjoining myocardial bundles. However, variations occur in which the swollen fibers present no approach to an orderly arrangement, running in all directions and forming irregular clumps and tangles. Figure 7 represents an early stage in the formation of this type of lesion. In another variant the points of fusion may become quite extensive and give the impression of large eosinophilic collagenous masses (Fig. 8). However, one can always observe these masses passing imperceptibly into isolated, more delicate strands.

Silver impregnation of the reticular framework discloses argentophilic fibers overlying the net of swollen collagen. We have not been able to convince ourselves that these argentophilic fibrils lie actually within the swollen substance (ground substance?). Van Gieson's stain regularly discloses an admixture of yellowish and reddish fibers which are in places continuous with one another. Again, it is extremely difficult to determine whether the picric acid-staining material represents a substance (ground substance) essentially different from the fuchsinophilic (fibrillar) material.

This swollen reticulum frequently takes on the tinctorial properties of fibrin. In their configuration, however, the fibers can easily

be distinguished from fibrin. Furthermore, it can readily be determined that these "fibrinoid" fibers are continuous with collagen fibers in the vicinity, where they show the characteristic staining for collagen.

Contrary to the views expressed by Klinge, the collagen framework is at times seen to be extensively broken down and granular. In places this granular material, resulting from considerable fusion of the collagenic fibers, may present appearances that merge imperceptibly with variants of the mosaic Aschoff body types to be described.

MOSAIC TYPE

This type of Aschoff body (Fig. 9) is the one most frequently encountered in the myocardium of the rheumatic heart. In contrast to the coronal types it consists of a more or less uniform distribution of collagen fibers and cells forming a mosaic pattern of the two components. Because of the variations in the proportions of collagen and cells, the size of the cells, the condition of the collagen and the manner in which the cells are lodged between the collagen masses, the mosaic forms may present a protean appearance. In one form the cells seem to be squeezed into the spaces or crypts between the collagen masses with the cytoplasm extending as streamers, which are sometimes extremely delicate, between the more dense and somewhat solid appearing, swollen eosinophilic collagen ground substance (Figs. 9 and 15). When these streamers form distinct cytoplasmic bridges between a number of the adjacent cells they tend to form large syncytial masses. In this mosaic type the cytoplasm is almost invariably deeply basophilic, the ragged edges are prominent, as is fragmentation of the cytoplasm and collagen. The argentophilic reticulum fibers are also quite prominent. There is a fairly even proportion between the owl-eyed and fibrocytoid nuclei. Pyknosis is not infrequently seen in this type.

In another variant of the mosaic Aschoff body (Fig. 10) the cells do not show the tendency to be compressed by swollen collagen masses, as in the variant previously mentioned. The cytoplasm is deeply basophilic and presents markedly irregular ragged edges. Both cytoplasm and collagen show considerable fragmentation. Indeed, the collagen may show such conspicuous granular degeneration as to be indistinguishable from the fused reticular type with granular

collagen previously described. A prominent silver-staining lattice is invariably present. It is this variant of the mosaic Aschoff body that has been loosely designated by a number of authors as the "typical" Aschoff body. Giant cells are not infrequently found. Owl-eyed nuclei occur with approximately the same frequency as that seen in the syncytial coronal type.

POLARIZED TYPE

This type of Aschoff body apparently represents a further stage in the metamorphosis of the Aschoff cells into fibroblasts. The topography of the lesion as a whole is that of a spindle-shaped or tapering disk-like mass representing many cells compressed between the myocardial bundles. The cells themselves vary from somewhat irregular elongated forms to spindle shapes, the difference probably depending on the maturity of the lesions. The irregular forms (Fig. 11) present ragged edges and are basophilic. The spindle-shaped cells (Fig. 12) also present a basophilic cytoplasm. The edges, however, are generally smoother and more sharply defined. The owl-eyed nuclei are still the predominating types but seem to occur somewhat less frequently than in the syncytial coronal or mosaic types. Pyknotic nuclei are seen with perhaps less frequency than in the types thus far described.

The collagen framework is generally delicate. While eosinophilic swollen fragments may be found the fibers show, as a rule, less swelling than in the other types. On the other hand, swelling and fragmentation may be occasionally quite conspicuous. The rather prominent argentophilic reticulum fibers show compression to conform with the spindle shape of the nodule as a whole (Fig. 13).

Another variety of the polarized type, which is not infrequently met with, may be called the "giant cell polarized type." In this form the Aschoff body shows a polarization into a rather short disk. The edges of the nodule do not generally taper off into a definite spindle shape. On section the appearance is that of rather large flattened giant cells, somewhat deserving the name of syncytial masses, because of cytoplasmic bridges between them with a rather scant collagenous framework. The cytoplasm is basophilic and the owl-eyed nuclei predominate.

FIBRILLAR TYPE

The last stage in the evolution of the Aschoff body before it becomes transformed into an interfascicular scar may properly be referred to as the fibrillar type (Fig. 14). The cells have definitely elongated themselves and approach the characteristics of fibroblasts. The extremely scant and generally still basophilic cytoplasm is arranged as blunt ends situated at either side of the elongated nucleus. The nuclei are now preponderatingly of the fibrocytoid variety, although owl-eyed forms may occur in fair numbers. The incidence of giant cells has become very small, whereas pyknotic nuclei are seen as frequently as in the mosaic types. The collagen has almost completely lost its eosinophilic swollen appearance and is represented by varying amounts of parallel, more or less isolated delicate fibrils with a rare swollen fragment. The argentophilic reticulum has now flattened itself out and from this stage on becomes less and less prominent.

Because of the fibrillar appearance of this Aschoff body type, it may be confused with the reticular Aschoff body. However, it is to be noted that the latter presents a swollen interlacing feltwork of eosinophilic collagen and that the cells are either round with deeply staining nuclei and scant cytoplasm or may appear considerably larger, ovoid, with a generous admixture of the three types of nuclei described. Giant cells may also occur. On the other hand, in the further evolution of the fibrillar Aschoff body, difficulties may be encountered in differentiating it from a somewhat cellular scar. However, the occasional presence of a giant cell and, here and there, of a ragged edge to the cytoplasm or blunt tip to the cell, and the presence of fibrocytoid, owl-eyed and pyknotic nuclei should render this lesion sufficiently characteristic to identify it as an Aschoff body.

DISCUSSION

The purpose of this descriptive classification is two-fold: first, to prepare the ground for further reports in which an effort will be made to ascertain the life cycle of the Aschoff body and to introduce as far as possible a time component into such studies; second, to reduce the controversies on the morphology of the Aschoff body to definite com-

mon denominators by showing that while these lesions occur in a variety of definitely recognizable forms they nevertheless retain each in themselves a sufficient number of characteristic features to justify their consideration as specific of rheumatic fever. The descriptive material in this report, which is largely devoted to separating the Aschoff body into definite categories, at the same time suggests a terminology that should be found useful for future studies. Moreover, this classification possesses sufficient elasticity to permit the addition of suitable descriptive terms (*viz.*, syncytial mosaic Aschoff body, giant cell polarized Aschoff body, and so on). Inasmuch as the lesions falling into these categories represent stages in an evolutionary process, it is to be expected that transitional forms will be encountered. In such cases a hybrid term may be employed. For example, Figure 15 illustrates a lesion that seems to be a transition from a coronal into a mosaic type. It may, therefore, be called a "coronal mosaic" Aschoff body. Figure 16 illustrates a transition between the polarized and fibrillar form and may be properly designated a "polarized fibrillar" Aschoff body.

While the earliest rheumatic lesions, where swelling of the collagen and leukocytic reaction are the only components, can by no means be considered specific of rheumatic fever, the types described in this classification, which represent further stages in the evolution of the lesion, seem to be absolutely specific, differing entirely from the lesions found, for example, in uncomplicated scarlet fever and subacute bacterial endocarditis, or in other inflammatory lesions of the heart. Simultaneously with the studies described here, an investigation was made of the argentophilic reticulum fibers, the cytoplasm, collagen, nuclear characteristics and the giant cells found in other lesions, such as in syphilis, tuberculosis, Hodgkin's disease, foreign body granulomas and Bracht-Wächter lesions, but there never was encountered unusual difficulty in discerning the Aschoff body from the lesions found in the above mentioned conditions.

The most frequently occurring and consistent common denominator in the structure of the Aschoff body is the basophilic cytoplasm with ragged edges. However, no single component can be designated as by itself forming a sufficiently characteristic feature to identify the Aschoff body. On the other hand, the polymorphous basophilic cells with their ragged edges, the swollen and fragmented collagen, the giant cells of the variety described in this report, the varying propor-

tions of somewhat large, owl-eyed, fibrocytoid and pyknotic nuclei, and the argentophilic network, form a sufficiently characteristic group of features to distinguish this lesion from those found in other diseases.

SUMMARY

The clinical histories and anatomical material from 70 cases of uncomplicated rheumatic fever with Aschoff bodies in the myocardium were investigated. A classification of Aschoff bodies is suggested, based on the appearance and distribution of the collagen, argentophilic fibers, cell cytoplasm and nuclei. This classification includes seven types of Aschoff bodies which apparently bear some relation to the life cycles of the lesions. Each type is described and is considered to possess sufficient characteristic features to identify it as an Aschoff body specific of rheumatic fever.

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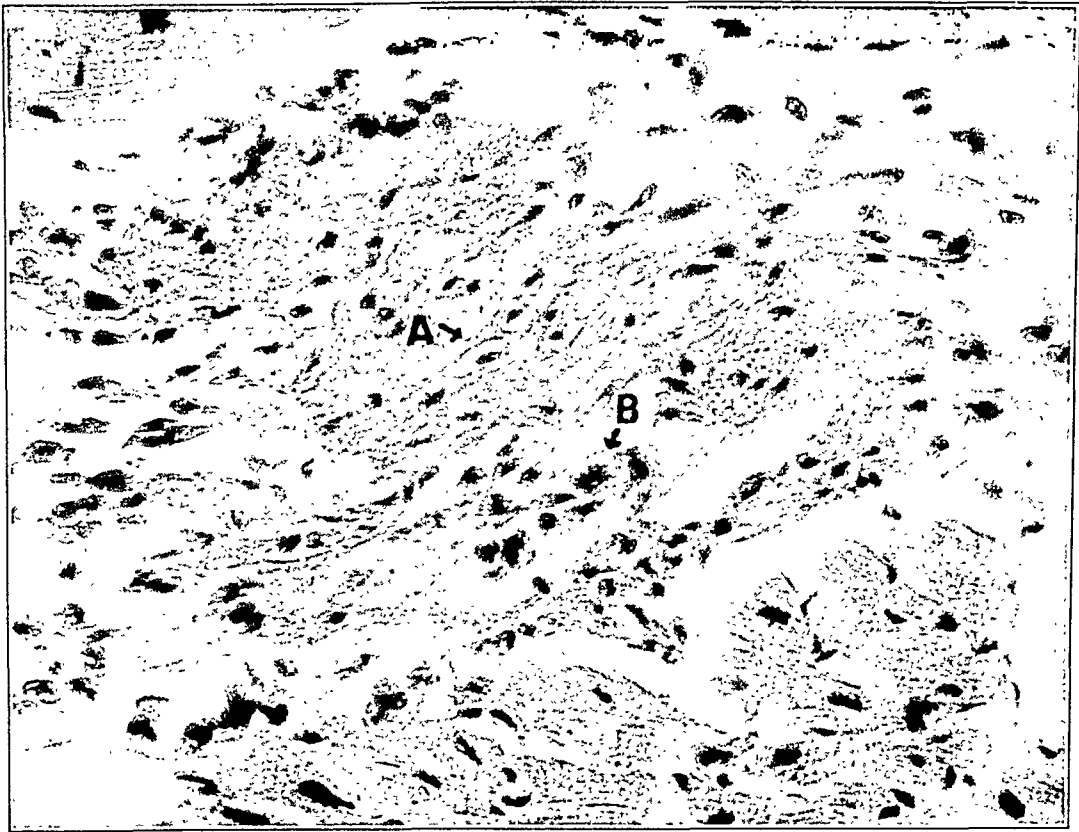
DESCRIPTION OF PLATE

PLATE 117

- FIG. 1. Small cell coronal Aschoff body. A, central swollen mass of collagen; B, cell with owl-eyed nucleus and increased cytoplasm; C, fibrocytoid nucleus. The majority of the cells present scanty cytoplasm.
- FIG. 2. Reticular Aschoff body. A, swollen collagen fibers forming interlacing network; B, cell with increased cytoplasm. Note that the collagen framework assumes a direction roughly along the lines of the myocardial bundles. The majority of the cells present scanty cytoplasm.



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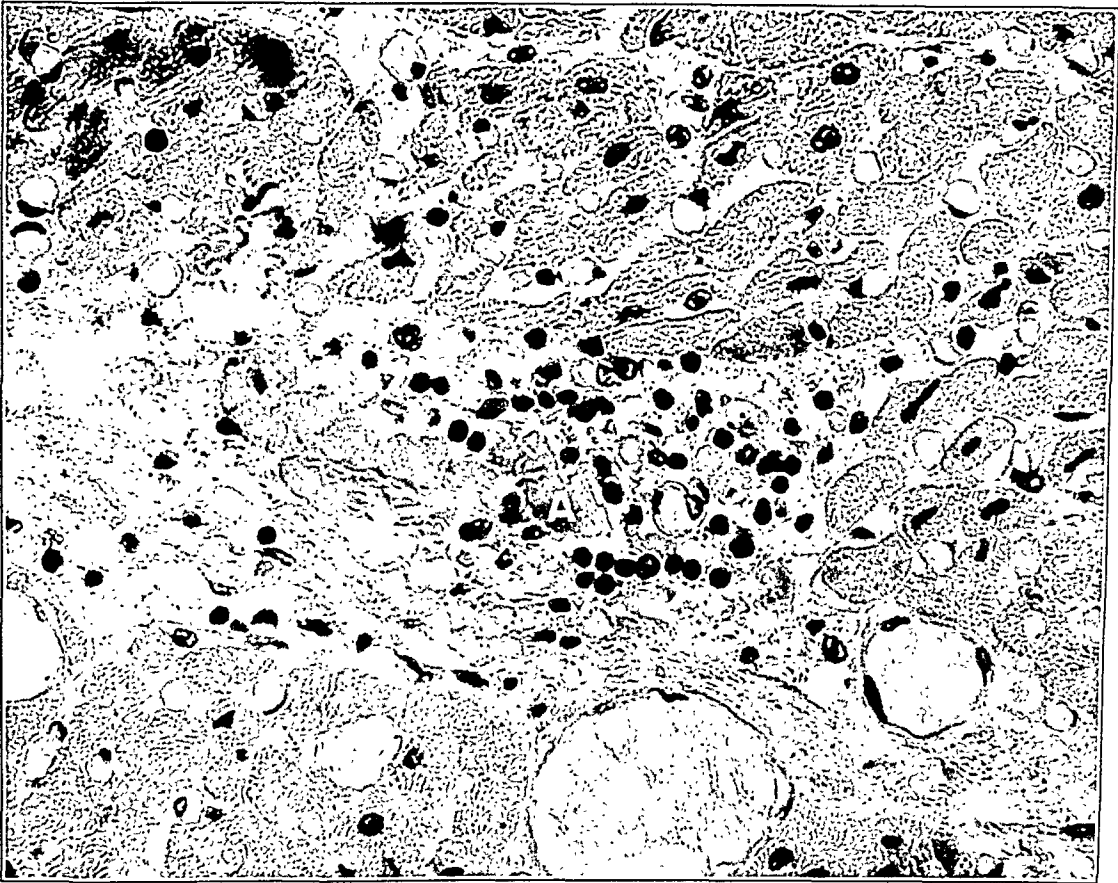


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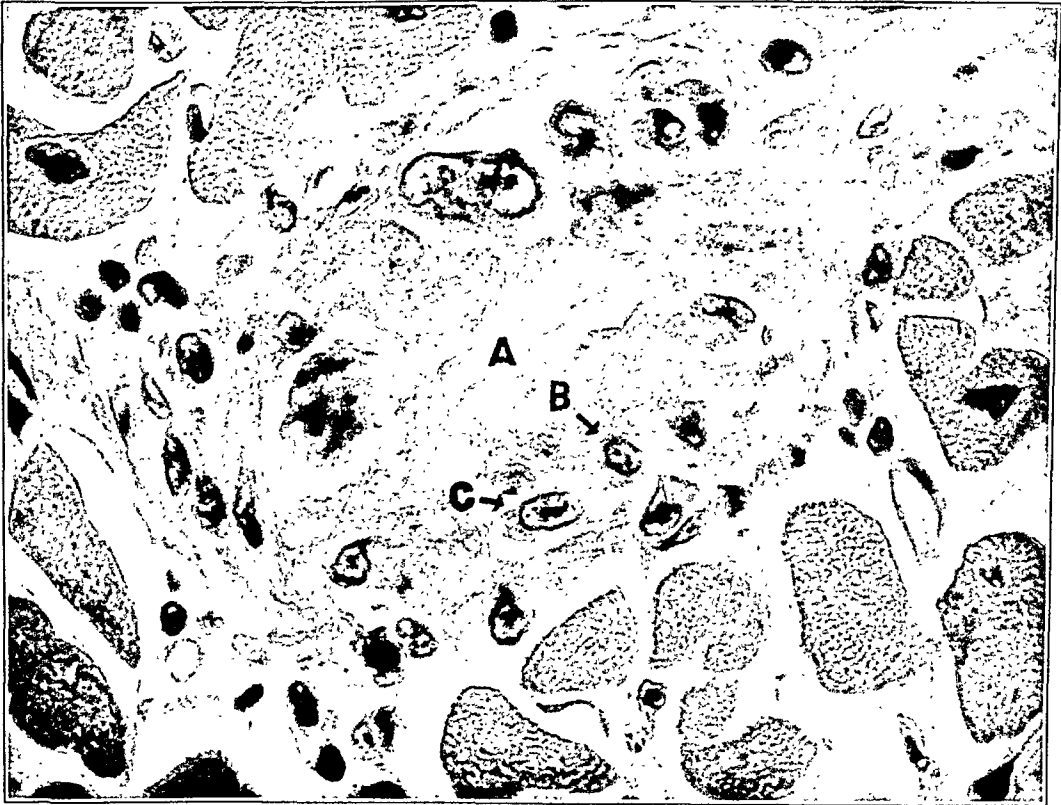
PLATE 118

FIG. 3. Stage preceding the formation of small cell coronal Aschoff body. A, swollen eosinophilic, somewhat fragmented collagen surrounded by small round cells.

FIG. 4. Large cell coronal Aschoff body. A, central swollen mass of collagen with some fragmentation; B, cell with fibrocytoid nucleus and indistinct cytoplasmic outline; C, cell with owl-eyed nucleus.



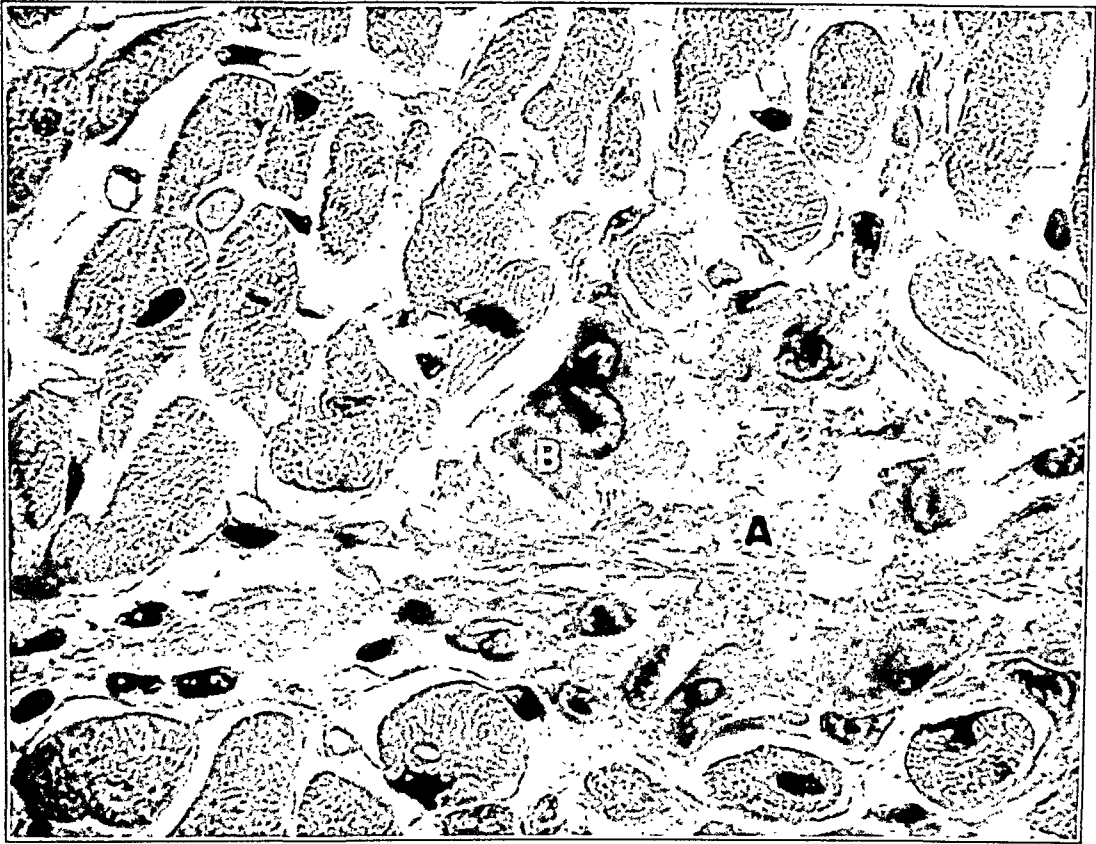
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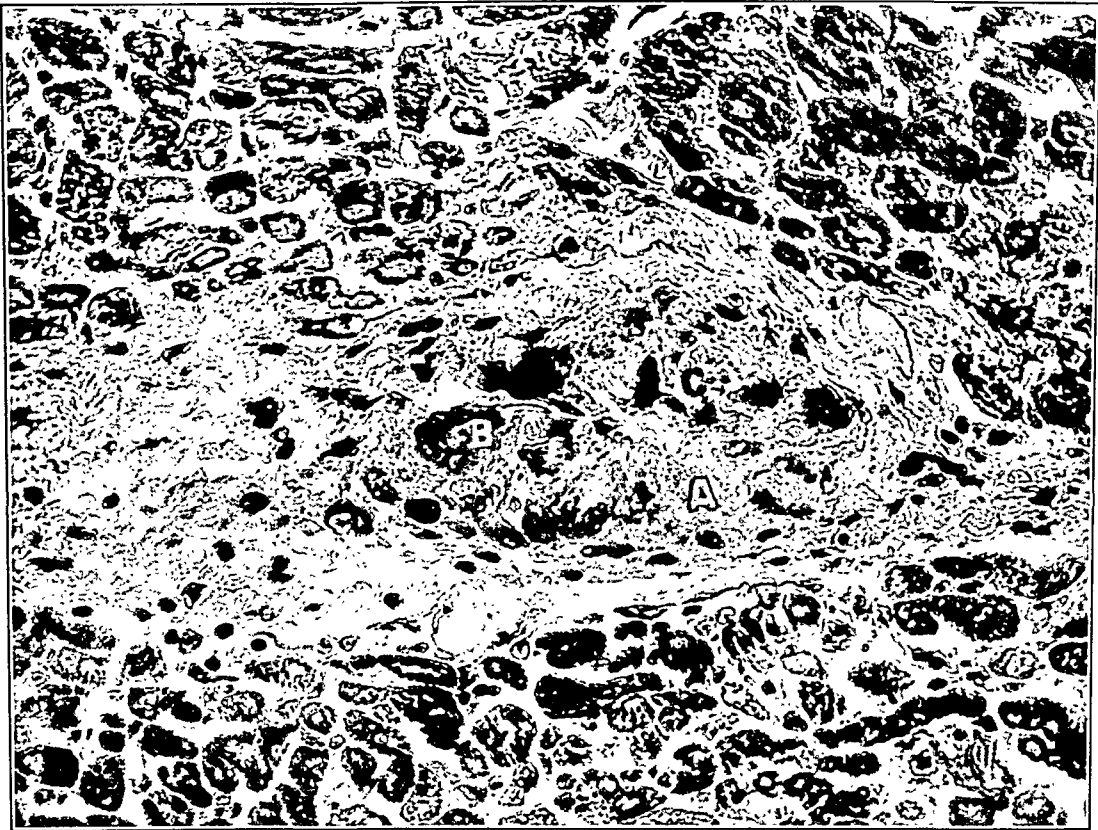
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PLATE 119

- FIG. 5. Large cell coronal Aschoff body. A, central swollen mass of collagen with some fragmentation; B, giant cell with ragged edges and owl-eyed nuclei. Note the marked basophilia of the cytoplasm (dark staining).
- FIG. 6. Syncytial coronal Aschoff body. A, portion of syncytial cytoplasmic mass showing extremely indistinct cellular outline; B, cytoplasmic mass with peripheral location of owl-eyed nuclei; C, masses of swollen collagen. Note small round cells in periphery of Aschoff body.



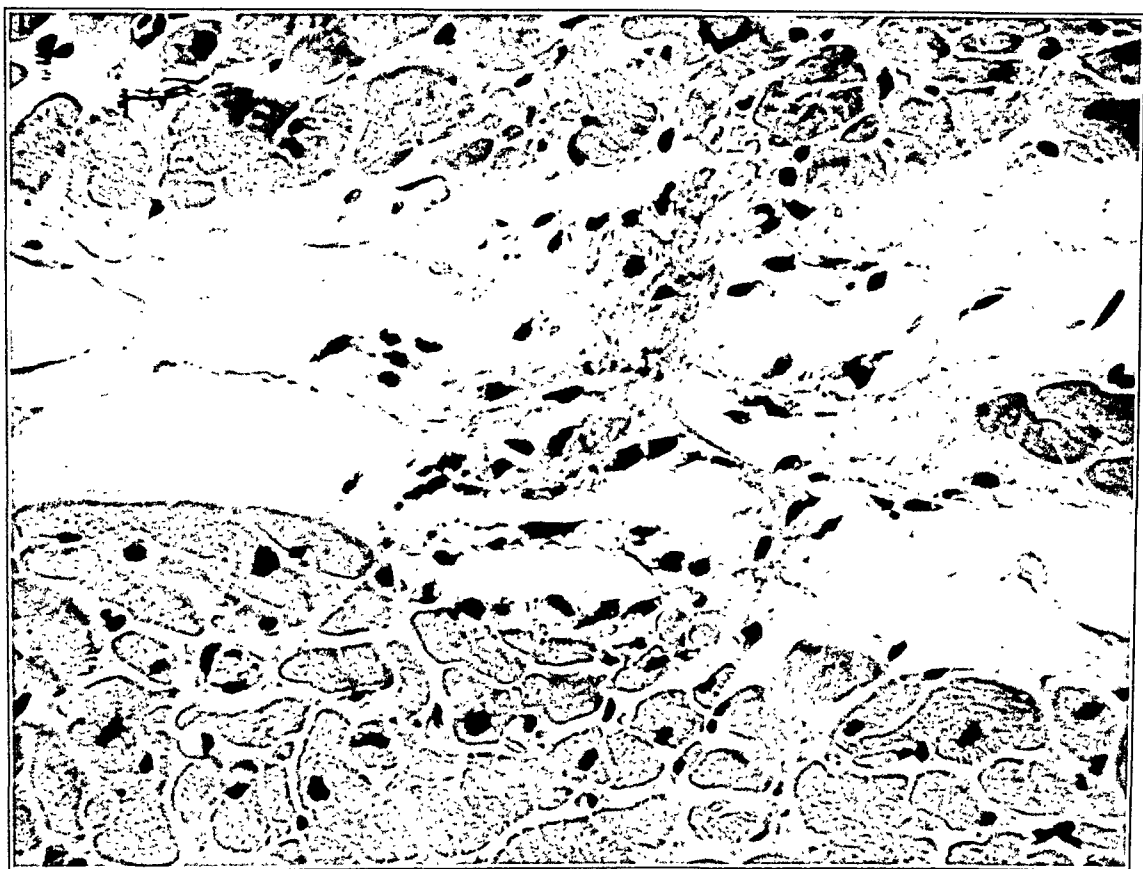
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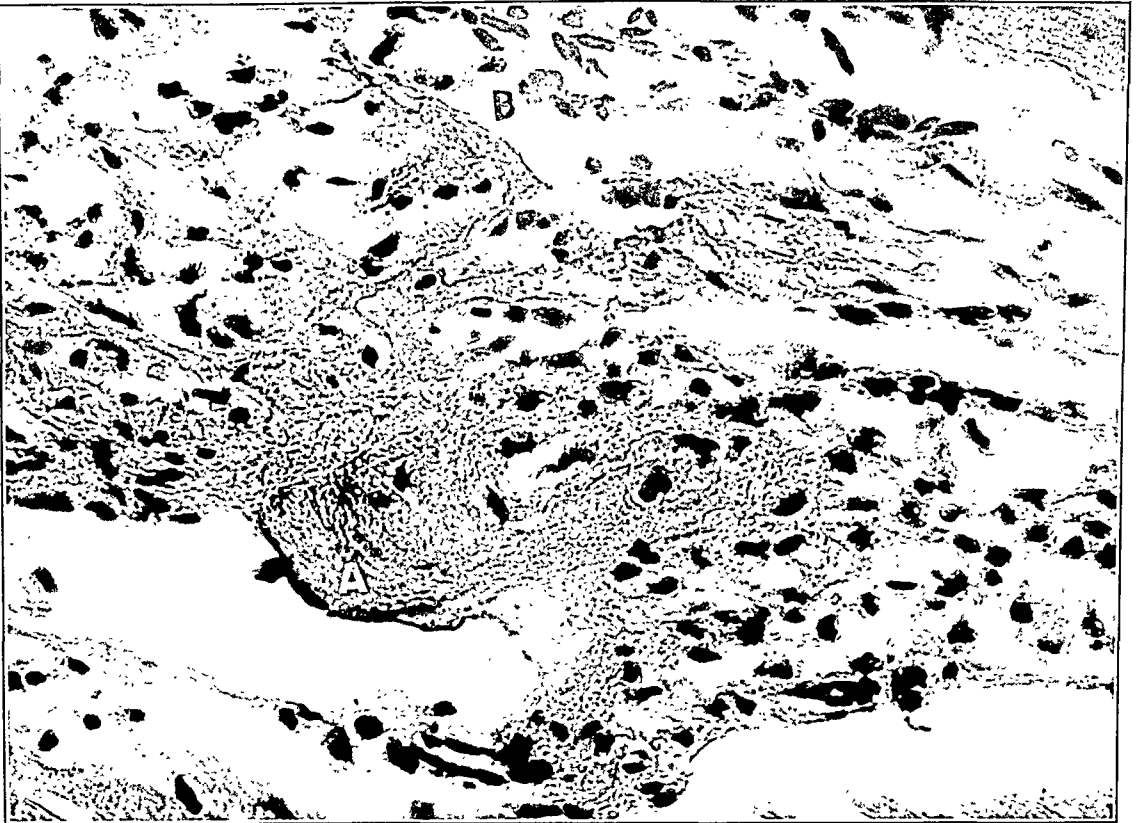
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PLATE 120

- FIG. 7. Early stages in the development of the reticular Aschoff body in the form of irregular clumps and tangles. The lesion cannot be considered specific until cellular evolution has reached the formation of owl-eyed, fibrocytoid and pyknotic nuclei with development of cytoplasmic basophilia.
- FIG. 8. Reticular Aschoff body showing (A) considerable fusion of collagen fibers.



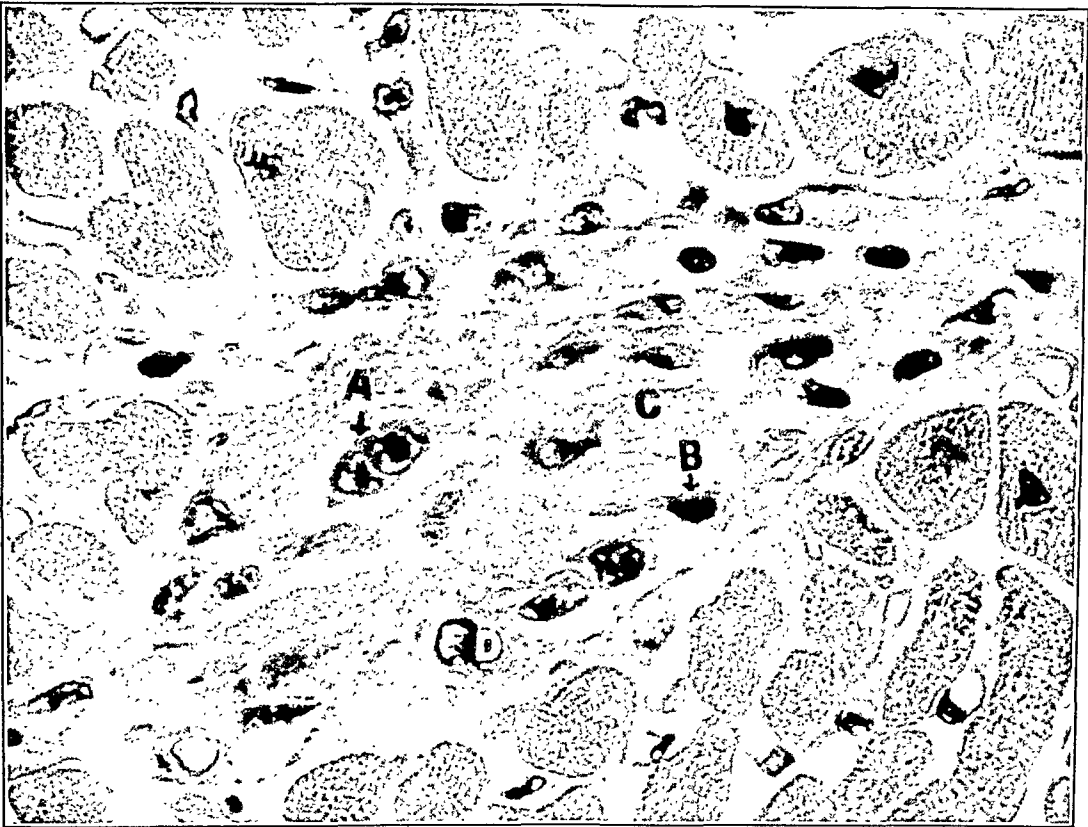
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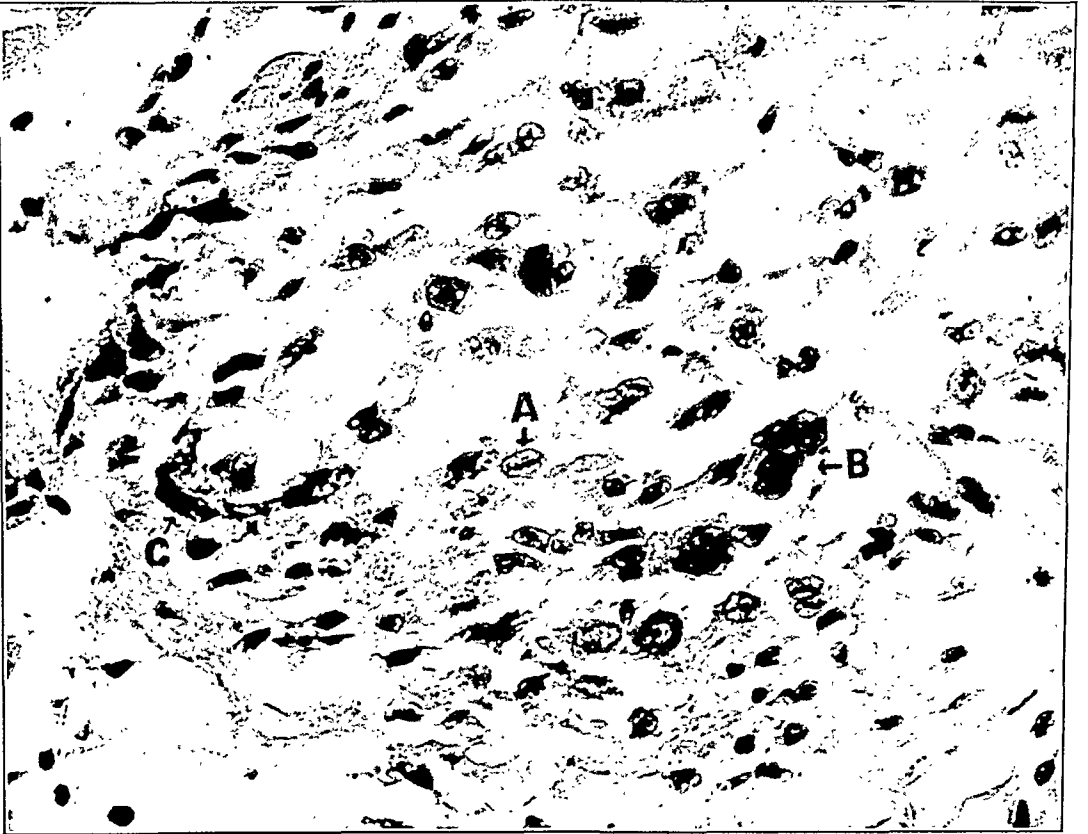
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PLATE 121

- FIG. 9. Mosaic Aschoff body of the more compact variety. Note the slight polarization of the cells. A, giant cell with irregularly outlined cytoplasm and two owl-eyed nuclei; B, cell with pyknotic nucleus and basophilic cytoplasmic streamer; C, swollen collagen; D, cell with extremely irregular ragged edges.
- FIG. 10. Mosaic Aschoff body of looser structure. Note fragmented appearance of collagen. A, fibrocytoid nucleus with radiating bar arrangement of chromatin; B, giant cell with owl-eyed nuclei; C, pyknotic nucleus.



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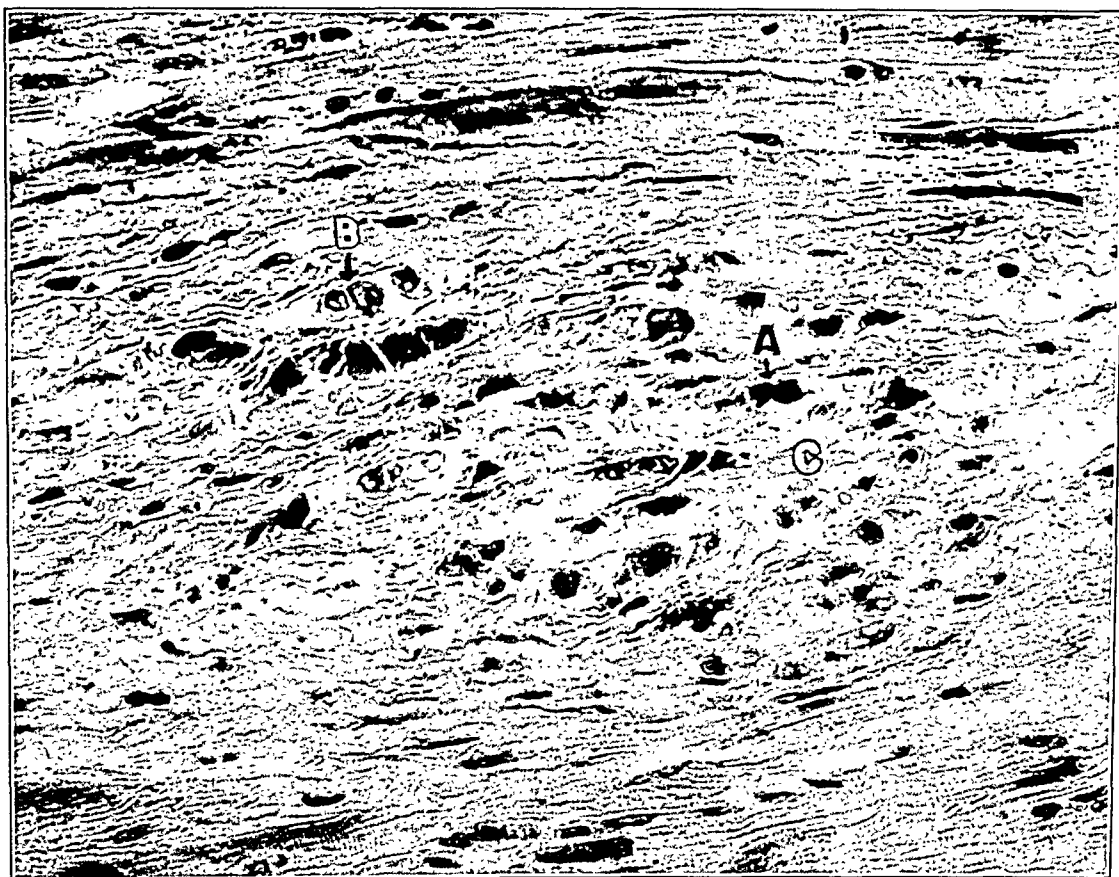


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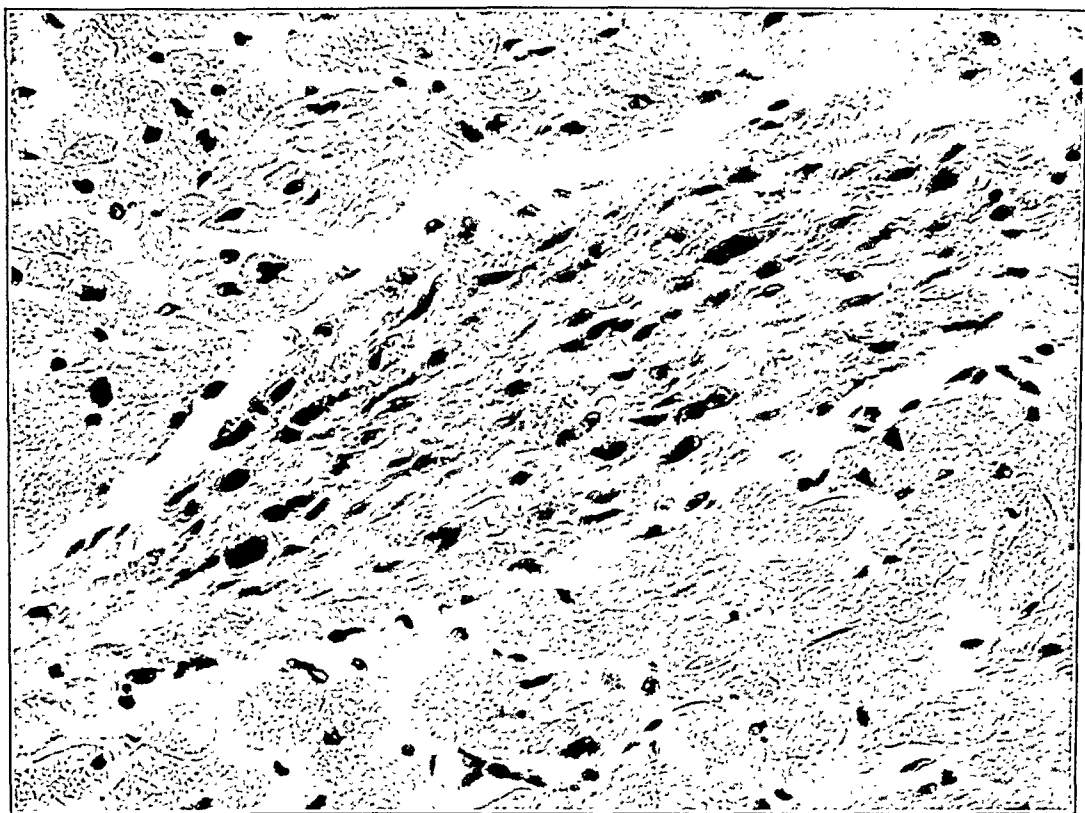
PLATE 122

FIG. 11. Large irregular cell polarized Aschoff body. A, pyknotic nucleus; B, giant cell with owl-eyed nuclei; C, fragment of swollen collagen.

FIG. 12. Polarized Aschoff body showing swollen and fragmented collagen and evolution of cells into spindle types.



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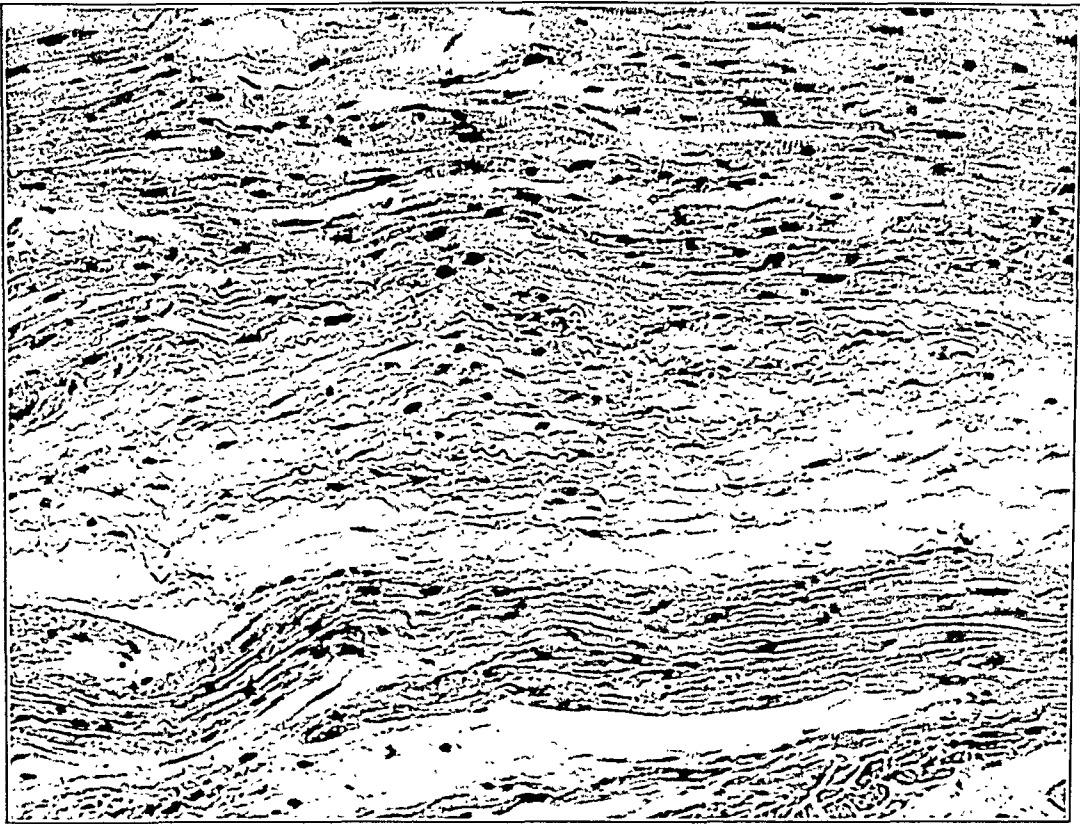
PLATE 123

FIG. 13. Argentophilic reticulum network in polarized Aschoff body.

FIG. 14. Fibrillar Aschoff body. Note the location of this lesion between the muscle bundles. Some of the cells still retain their basophilic cytoplasmic streamers. The nuclei still occur in the owl-eyed, fibrocytoid and pyknotic forms.



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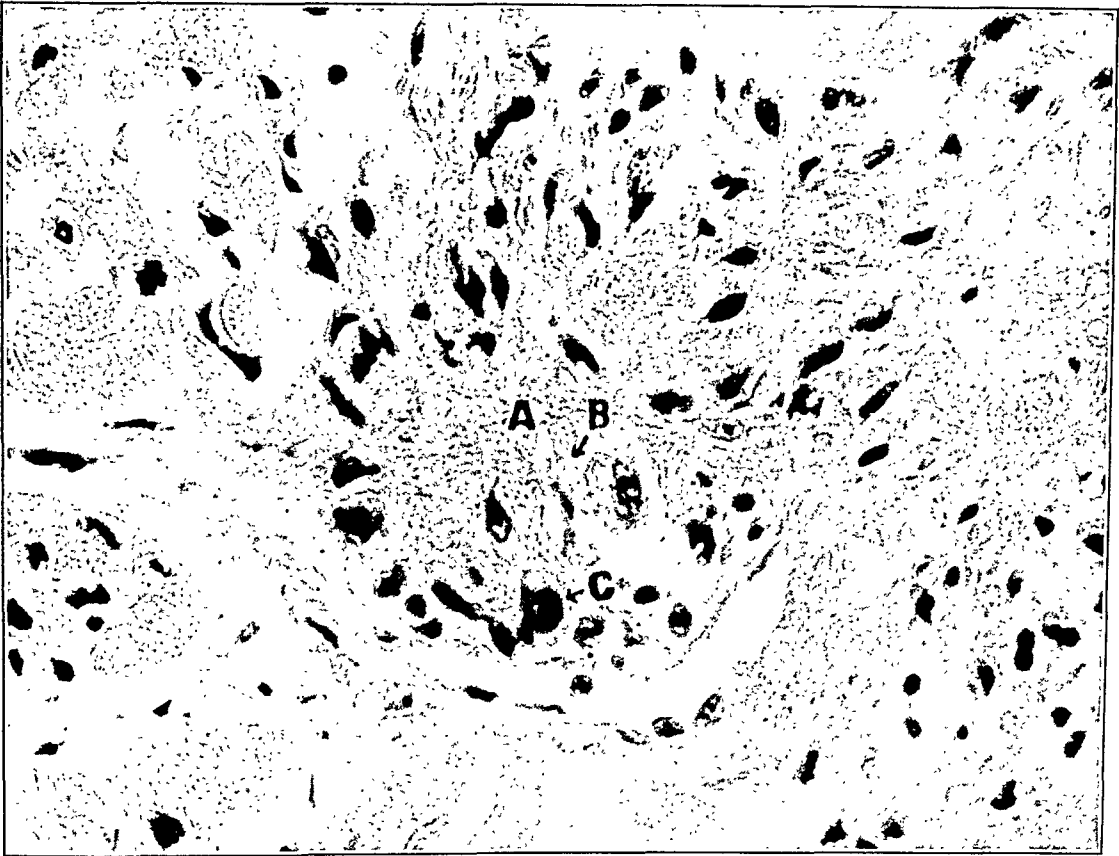


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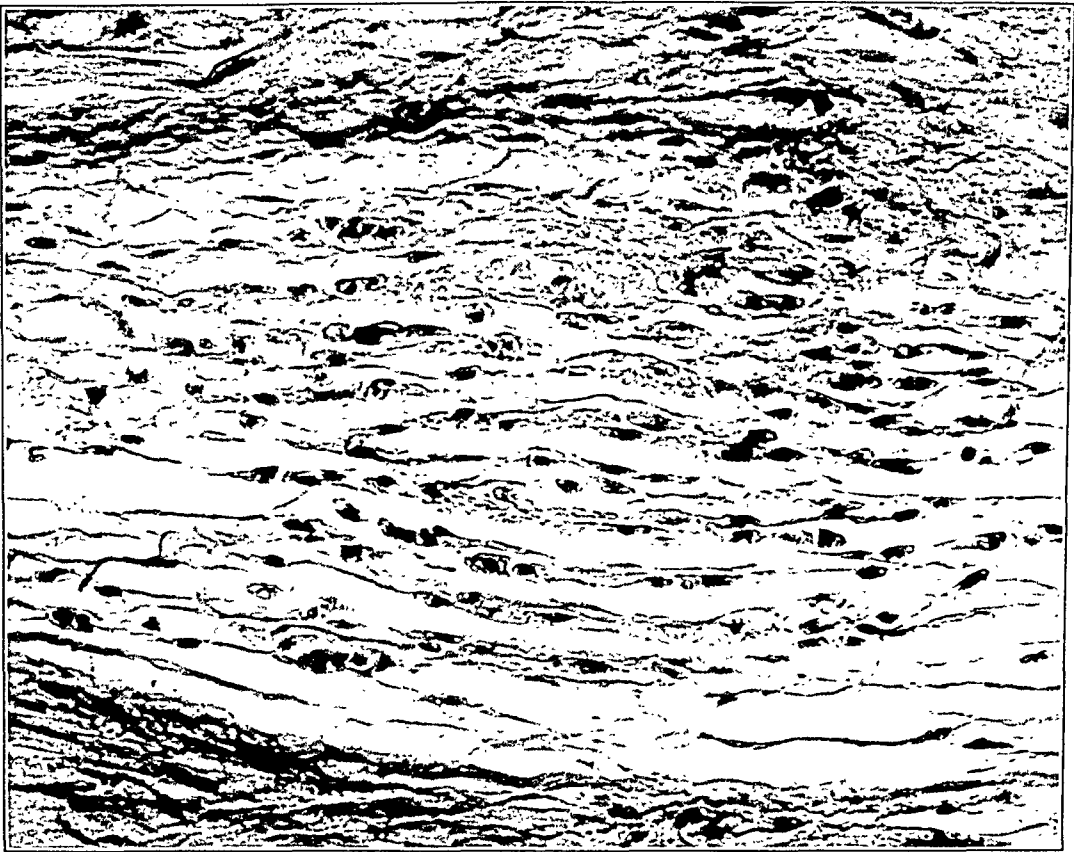
PLATE 124

FIG. 15. Coronal mosaic Aschoff body. A. central swollen collagen; B, cytoplasmic streamer from a cell penetrating the collagenous mass; C, large cell with pyknotic nucleus.

FIG. 16. Polarized fibrillar Aschoff body. Note marked elongation of cells with development of delicate collagen fibers.



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STUDIES ON THE MYOCARDIAL ASCHOFF BODY *

II. LIFE CYCLE, SITES OF PREDILECTION AND RELATION TO CLINICAL COURSE OF RHEUMATIC FEVER

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This report represents an attempt to throw further light on the nature of the myocardial Aschoff body, its life cycle, sites of predilection, frequency of occurrence, and its possible relation to the clinical course of rheumatic fever. In a previous publication ¹ we have shown that the Aschoff body occurring in the heart should be considered apart from the corresponding rheumatic lesions found in other tissues, such as skin, diaphragm, tendinous insertions, and so on, inasmuch as the myocardial lesions present specific characteristics that are either lacking in the other sites or have been insufficiently studied as yet. Furthermore, within the heart proper the Aschoff bodies appear to present characteristics that are definitely influenced by their site. Thus, the subendocardial, left auricular endocardial and perivascular lesions occur in a compressed form which modifies markedly the topography of the evolutionary stages. Within the looser milieu of the interstitial connective tissue between the myocardial bundles the Aschoff body passes through the various stages of its life cycle relatively unhampered by dense fibro-elastic tissue and, accordingly, presents its more characteristic and fully developed metamorphoses. For these reasons the studies presented here will concern the Aschoff bodies situated between the myocardial bundles — lesions that we have referred to as myocardial Aschoff bodies.

Despite reports, particularly in recent years, in which some doubt is expressed concerning the specificity of this lesion in its relation to rheumatic fever, a review of the literature dealing with the development of our knowledge concerning the Aschoff body reveals sufficient evidence to warrant the definite assumption that this lesion does not occur in the myocardium if rheumatic fever, past or present, can

* Aided by a grant from the Lucius N. Littauer Foundation.
Received for publication April 26, 1934.

safely be ruled out of consideration. We shall assume, therefore, for purposes of this study, that it is absolutely specific of this disease.

It is pertinent to this report to mention that one of the reasons for doubting the specificity of this lesion has been the fact that the Aschoff body assumes allegedly protean forms and that one cannot, therefore, speak of a "typical Aschoff body." In a paper presented before the American Association of Bacteriologists and Pathologists² we have shown that the supposed protean appearance of the Aschoff body is due to the fact that the published descriptions have not infrequently referred to different stages in the life cycle of the lesion and that, on the contrary, there is a remarkable constancy in the appearance that these inflammatory nodules assume. We have been able to show¹ that each stage in the development of the lesions apparently presents such a uniformity in characteristics that the Aschoff bodies in their specific forms (the earliest stages — collagen swelling and mesenchymal cell proliferation — cannot be considered a specific picture) may be classified into seven relatively clear-cut and easily recognizable types. Occasionally hybrid forms may be encountered, which undoubtedly represent transitions from one type to another. In order that the argument throughout this report may be followed more easily we shall first present a short description of the seven types of Aschoff bodies found in the myocardium. A discussion on the classification of these lesions, together with a fuller demonstration of their histological characteristics, will be found elsewhere.¹

ABSTRACT OF CLASSIFICATION OF ASCHOFF BODIES

1. *Small Cell Coronal Type:* This consists of a central swollen mass of eosinophilic collagen surrounded by round or oval cells somewhat larger than lymphocytes with a delicate mantle of basophilic cytoplasm which presents a sharply defined edge. The nuclei are of the three types that have been described by us as common to all types of Aschoff bodies in which, however, they occur in different proportions: (1) fibrocytoid, (2) owl-eyed, and (3) pyknotic. Giant cells are occasionally seen. This type of Aschoff body shows the beginnings of a network of argentophilic reticulum fibers.

2. *Large Cell Coronal Type:* This differs from the small cell type in the following respects: the cytoplasm is more abundant, the edges

may be diffuse and ragged, giant cells are seen somewhat more frequently and the network of argentophilic fibers is more prominent.

3. *Syncytial Coronal Type*: In this type the giant cells assume the form of large syncytial basophilic masses with ragged edges. These masses arrange themselves around generally small, centrally located fragments of swollen collagen. Owl-eyed nuclei are quite prominent and tend to assume a peripheral situation within the cytoplasmic masses.

4. *Reticular Type*: This consists of a feltwork of interlacing, swollen collagen fibers which frequently fuse at their points of intersection. Within the meshes of this network there are to be found small cells, round or ovoid, with scant basophilic cytoplasm. Occasionally larger cells with a scattering of owl-eyed, fibrocytoid and pyknotic nuclei may be found. Giant cells may be present. Variations occur in this type in which the collagenous meshwork may be extremely irregular, or where fusion may go on to the extent that larger collagenous masses are produced.

5. *Mosaic Type*: This type is characterized by a fairly regular intermingling of cells and collagen. The cells are abundantly cytoplasmic, deeply basophilic, somewhat fragmented and possess ragged edges. The collagen is swollen, eosinophilic and often fragmented. The cells may be squeezed between the crypts of the collagen masses and connected by delicate cytoplasmic streamers, or the mosaic may be of looser structure, in which case the cells tend to be extremely irregular in shape. The argentophilic fibers occur in the form of a net.

6. *Polarized Type*: In this type the cells begin to assume a spindle shape. They may still be somewhat irregular and elongated or they may present a somewhat smoother contour and still retain the basophilia of the cytoplasm. The entire collection of cells takes on a definite direction within the planes of the myocardial bundles. The argentophilic network is somewhat compressed.

7. *Fibrillar Type*: This is a stage that precedes complete metamorphosis of the cells into fibroblasts. As a consequence the cytoplasm is extremely scant, occurring at times as somewhat blunt basophilic knobs at either end of the much attenuated cell. The nuclei are largely fibrocytoid. Giant cells are infrequent. The collagen occurs predominantly in the fibrillar form. The argentophilic fibers are rapidly disappearing.

As to the origin of the cells, we have already stated that it is safest to assume that they arise from mesenchymal elements, understanding by this term an ultimate derivation from the mesenchyme which may or may not have passed through a differentiation into mature cell types such as lymphocytes, histiocytes or fibroblasts. It is also accepted by many observers that injury to the collagen framework of the heart plays the most conspicuous rôle in the determination of these lesions. As a consequence Aschoff bodies occur at those sites where the collagen is found in the greatest amount, *viz.*, in the planes between the myocardial bundles, in the fibro-elastic tissue around blood vessels and in the endocardium. It is the primary lesion to this collagen, with the subsequent development of cellular reaction of a specific type, that gives rise to the Aschoff body.

The first question with which we shall deal is whether a given heart presents a relatively uniform development of Aschoff bodies in respect to their type. Obviously, if this is not the case, any attempt to introduce a time component in the development of the life cycle of this lesion becomes fraught with such difficulty as to make it an apparently hopeless task. Furthermore, such a state of affairs would lend support to the argument that the Aschoff body may arise in a variety of forms. It may be said at once, therefore, that in an examination of 70 hearts possessing Aschoff bodies we have found that a given section usually presents one type of Aschoff body, occasionally two and rarely three. Furthermore, one frequently encounters in the heart a remarkable uniformity in Aschoff body types, particularly if the specimen is obtained from a patient dying in a first attack, or where the attack has taken place a long time (2 years or more) subsequent to a previous attack. This consistency in the structure and, therefore, in the age of the Aschoff body permits of the reasonable assumption that a given crop of lesions may be timed from the onset of a given attack; and since they reach the same evolutionary stages in development, as judged by their appearance at the time of death, it would seem that these lesions pass through an orderly and probably similar series of progressive changes.

LIFE CYCLE OF THE ASCHOFF BODY

Several of the published reports make mention of the ultimate fate of the cells concerned in the formation of the Aschoff body. Thus, Aschoff,³ in 1904, believed that the cells eventually transform them-

selves into fibroblasts. Geipel ⁴ in 1905 observed the development of the lesion in the 5th to 6th week after the onset of the illness, the formation of giant cells and the swelling and later fibrillar change of the ground substance. Takayasu ⁵ in 1909 described what appears to have been a mosaic Aschoff body occurring 3 months after the onset of the illness. An excellent contribution along these lines was made by Talalajew ⁶ in 1929, who divided the evolution of the Aschoff body into three phases: (1) exudative, found at the end of the 2nd or during the 3rd week of the illness; (2) proliferative, occurring in the 2nd or during the 3rd month and lasting, at times, 6 months; and (3) sclerotic, appearing during the 2nd month and lasting, at times, 6 months. In 1930 we ² presented evidence of many evolutionary phases in the development of the Aschoff body through coronal, mosaic, polarized and fibrillar stages. While a sequence of events was suggested, no intimation was made of the actual time component of each phase. Additional information concerning the time it takes to reach the several evolutionary stages of the Aschoff body was formulated by Klinge ⁷ and his associates in a series of papers (1929 to 1933). According to this work, at the end of the 2nd week of the illness this lesion is represented by swelling of the collagen fibers and increase of connective tissue and wandering cells, with the presence of occasional giant cells. After the 4th week many swollen and multinucleated cells are found arranged either in rosette form around swollen collagen or dispersed throughout it. From this period on involutionary changes take place through the disappearance of the giant cells and "fibrin" and the development of connective tissue cells.

Before proceeding with our own observations on the life cycle of the Aschoff body it may not be amiss to mention the criteria employed by us to determine the sequence of events and the time factors in the cycle. As mentioned before, 70 hearts, each presenting Aschoff bodies in the myocardium, were studied. These were described grossly, particular care being taken to note the extent of valvular damage. The specimens were generally fixed in formol-saline,* and blocks were cut and stained by a number of methods designed to demonstrate changes in the collagen, tinctorial properties of the cytoplasm, details in nuclear structure and presence of

* For a more detailed description of the methods employed see Gross and Ehrlich.¹

bacteria, fibrin, argyrophilic reticulum and elastic tissue. The case records from all this material were studied in order to determine the clinical course and particularly to set the time of onset of the last attack.

Apart from the clinical records we attempted to fix the onset of the attack by the extent of the valvular damage, as determined by its macroscopic and microscopic appearance and by the histology of the left auricular lesion, when present. The most reliable guide, however, appeared to be the state of the collagen. In common with a number of observers we were impressed with the fact that the collagen was the first to show damage in the form of swelling and the assumption of eosinophilic properties. The earliest appearance of swelling was, therefore, taken as confirmatory evidence that we were dealing with the beginning of the cycle. In this stage (early phases) the reticular and small cell coronal Aschoff body type predominated. On the other hand, it seemed equally reasonable to assume that the late phases of the cycle were associated with the appearance of fibroblasts (metamorphosis of the Aschoff cells) and the transformation of the swollen collagen into the delicate fibrillar form. These stages were associated with the polarized and fibrillar forms of Aschoff bodies. Moreover, the clinical records confirmed the fact that these were late stages in the evolution of the attack. There remained, therefore, the large cell coronal, syncytial coronal and mosaic forms which, both by the nature of the collagen and on structural considerations, appeared to fall naturally in between these extremes in the age of their development (middle phases). At best, of course, these can be considered no more than an approximation to what actually happens in the human heart.

The material least subject to criticism on which it is permissible to attempt the reconstruction of the stages through which the Aschoff body passes is represented by cases where the individual died in his first attack, and where the time of onset of the disease is definitely known. For this purpose we had available 9 cases, all children who died from 2 to 13 weeks after the onset of the rheumatic fever symptoms, *e.g.*, joint pains and temperature. It is to be noted that in a discussion of the time component of these lesions we shall date their age from the onset of the rheumatic phenomena — disregarding what may be considered a prodromal period, namely, the possible preceding attack of sore throat, scarlet fever, and so on. It

is generally believed that this prodromal period may vary from several days to 4 to 5 weeks. A study of these "first attack" cases indicates that the earliest lesions are of the reticular type, and that fibrillar forms are not found by the end of the 13th week, at least in the limited number of cases available for study. On the other hand, a careful selection of material from cases where the individual suffered from more than one attack presents clear-cut evidence that in these clinical groups the initial lesions may be represented by reticular, as well as small cell coronal forms. Furthermore, an opportunity is thus made available to study the development and time factors of the fibrillar form.

As indicated in a previous publication, it seems fairly certain that the earliest stages consist of swelling, eosinophilic metamorphosis and a certain amount of fusion of the collagen fibers with, *pari passu*, proliferation of the mesenchymal elements. These non-specific early stages may occur in two forms which eventually develop into the coronal and reticular Aschoff body types.

Before entering into a description of the sequence of events that represent the life cycle of the Aschoff body it is of value to classify our material into four clinical groups representing the course taken by the rheumatic fever process in our 70 cases which came to autopsy and presented Aschoff bodies in the myocardium. This classification was undertaken because it appeared that the clinical course of the disease modifies to a certain extent the evolutionary process of the Aschoff body in a given case.

CLINICAL CLASSIFICATION OF RHEUMATIC FEVER MATERIAL

GROUP 1. Cases where the individual died in a first attack.

GROUP 2. Cases where one attack occurred prior to the final fatal recurrence.

GROUP 3. Repeated attacks with death during an acute recurrence.

GROUP 4. Cases where death was caused by decompensation without clinical evidence of a final recurrence. Some of these cases had no previous history of rheumatic fever.

Early Phases: In the "first attack" cases (Group 1), as well as in the other groups where the collagen in the interstices of the myo-

cardium exists in a somewhat loose fibrillar form, the reticular Aschoff body apparently represents the earliest specific lesion. In addition, however, in Groups 2, 3 and 4 the early lesion is also represented with about the same frequency by the small cell coronal Aschoff body type, both types existing side by side in the same blocks taken from the myocardium. The fact that the Group 1 cases show reticular forms exclusively (or, perhaps, predominantly) suggests that the form taken by the initial lesion is apparently influenced not only by the fact that the small cell coronal form begins around more compact collagen, but possibly also by the altered reactivity of the individual, due to the fact that he has already suffered an initial attack. These early stages (early phases) are found in from 2 to 4 weeks after the onset of the disease. Since we did not have any cases where the individual died sooner than 2 weeks after the onset of the disease we have been unable to determine precisely how soon the earliest specific lesion may be found before this time.

Middle Phases: Depending upon whether the earliest lesion is the reticular or small cell coronal Aschoff body type, the nodule may develop in one of two main directions during its middle phase. The reticular form rapidly shows increase in the size of the cells, during which time the collagen may either become more delicate, or fuse and undergo granular degeneration. If the collagen becomes relatively inconspicuous and delicate the cells elongate themselves and there develops the picture of the large irregular cell polarized type. If the collagen undergoes fusion and granular degeneration the resultant picture may be one indistinguishable from the mosaic type with necrotic collagen. This process apparently takes place between the 4th and 13th week after the onset of the illness.

The small cell coronal Aschoff body transforms itself into the large cell variety by swelling of the cell cytoplasm. These swollen cells soon appear to penetrate into the collagenous central mass and eventually permeate it in such a manner as to form the mosaic Aschoff body. If the intercellular collagen undergoes granular degeneration a picture is produced that simulates the corresponding form already described as derived from the reticular Aschoff body. The stage during which the cells begin to permeate the central collagenous mass can be referred to as the coronal mosaic Aschoff body. In some cases the cells of the large cell coronal Aschoff body undergo amitotic division, fusion and enormous enlargement, form-

ing huge syncytial masses which surround the relatively insignificant collagenous central portion (syncytial coronal Aschoff body). This lesion was found most frequently in the "first attack" group and generally appeared during the 9th week after the onset of the attack. Eventually, the syncytial masses disintegrate and produce a picture indistinguishable from the mosaic forms. As in the development that takes place from the reticular Aschoff body, these middle phases in the evolution from the small cell coronal lesion also occur between the 4th and 13th week after the onset of the disease.

Late Phases: The large irregular cell polarized Aschoff body, as well as the mosaic Aschoff body, whether derived from the reticular or small cell coronal lesion, now begins to show elongation of the cells to spindle forms. The cytoplasm still retains its basophilia but the outlines become sharp. The collagen becomes scanty and there is thus developed the polarized Aschoff body. Apparently, these lesions generally appear from the 9th to the 16th week after the onset of the disease. It is seen, therefore, that no matter which of the two initial lesions subsequently develops through the evolutionary stages of the Aschoff body, the lines of development apparently ultimately converge into the polarized forms.

From this point on, the spindle cells apparently transform themselves into fibroblasts. Delicate collagenous fibrils, which may ultimately fuse into dense collagenous bundles, appear between the cells. For some considerable time, however, the cells still retain rather blunt basophilic knobs of cytoplasm at either end of the elongated nucleus. Furthermore, whereas giant cells become extremely scarce and the nuclei become largely fibrocytoid, there are still to be seen a sufficient admixture of owl-eyed and pyknotic nuclei which, together with the peculiarity of the cells, distinguish this lesion as specific of rheumatic fever. We have designated these lesions as fibrillar Aschoff bodies. They apparently occur some time after the 13th week following the onset of the illness. The final stage in the evolution of this specific inflammatory lesion is the complete metamorphosis of the fibrillar Aschoff body into scar tissue which lies rather characteristically between the muscle bundles.

Figure 1 illustrates diagrammatically the lines of development of the Aschoff body, starting from the reticular and small cell coronal lesions and ending in the fibrillar type. An indication is given of the time component, although it must be realized that the attempt which

we have made at timing the development of these lesions represents an average arrived at from a study of relatively few specimens. There can be no doubt that considerable variations occur in the tempo, but this can be determined only from a much larger series of cases.

RELATION OF ASCHOFF BODY TYPE TO CLINICAL COURSE OF RHEUMATIC FEVER

In examining the types of Aschoff bodies found in the four clinical subdivisions of rheumatic fever outlined above several observations seemed to be worthy of note. Thus, the small cell coronal lesion was not found in the first group. This may have been due to the limited material available. On the other hand, as stated before, it may represent a difference in the reactivity of these "first attack" cases from the other clinical types. The large cell coronal, syncytial coronal and mosaic types were found more frequently in this group than in the other three. Fibrillar forms were not found. This may be due to the fact that death occurred within 13 weeks after the onset of the illness in the cases that comprised this group. In the second clinical group the incidence of small cell coronal and fibrillar types, as the initial lesions, was about equal. Mosaic forms occurred frequently, perhaps, however, slightly less often than in the first group. Fibrillar forms occurred with moderate frequency and large cell coronal types were relatively infrequent. The third group showed a further decrease in the incidence of mosaic forms, the lowest incidence of fibrillar forms found in any group with the exception of Group 1, and the highest incidence of polarized forms found in any group. Inasmuch as these two types of lesions are to a certain extent reciprocals of one another this observation does not indicate a fundamental difference in this group. No reticular forms were found in the fourth group, which otherwise showed approximately the same incidence of lesions as found in the second group. The conspicuous points in these observations appear to be the absence of small cell and fibrillar Aschoff bodies in Group 1, the high incidence of mosaic forms and the relatively high incidence of large cell coronal and syncytial coronal forms in this group, and the absence of reticular lesions in Group 4.

INCIDENCE AND DISTRIBUTION OF ASCHOFF BODIES IN
THE MYOCARDIUM

The figures quoted in the earlier literature (see Clawson¹⁰) on the incidence of Aschoff bodies in the hearts of patients dying from rheumatic fever are considerably higher than those obtained by later investigators. The reason for this discrepancy undoubtedly lies in the fact that the earlier workers chose active cases on which to make their studies. On the other hand, the figures recently published have also been high, probably because of better recognition of these lesions and a more thorough search for them. In comparing the reported incidence it must be remembered that the type of material studied plays an important rôle. Unless the proportion of acute and chronic cases studied is indicated no true comparison can be made. Furthermore, as will be shown later, the number of blocks studied and, more particularly, the sites from which these blocks have been taken, will materially influence the results obtained. Of the more recently published figures it is of interest to note that Aschoff bodies were found in the myocardium in 18 of Libman's⁸ 56 cases (32 per cent), 20 of Kugel and Epstein's⁹ 24 cases (83 per cent), 31 of Clawson's¹⁰ 50 cases (62 per cent), 24 of McClenahan and Paul's¹¹ 28 cases (85.7 per cent), 42 of Gross, Antopol and Sacks'¹² 79 acute and chronic cases (53 per cent), and in 60 of Thayer's¹³ 64 cases (93.7 per cent).

In our present statistics, which are based on a study of the standardized blocks, Aschoff bodies were found in approximately 59 per cent of 161 hearts showing evidence of rheumatic infection, past or present, and in 90 per cent of hearts in cases that showed evidence of activity, clinically or pathologically. The clinical evidence of activity can be considered to consist of joint pains, choreic manifestations and fever. The anatomical evidences of activity consist of fresh verrucous lesions, fresh pericarditis and acute inflammatory phenomena in the myocardium, valve rings and valve leaflets. The question of the relation of activity to myocardial failure and the incidence of these phenomena during the first eight decades of life have been studied by Rothschild, Kugel and Gross,¹⁴ who were able to show that during the first five decades of life myocardial failure is closely paralleled by activity in the myocardium.

Classifying our rheumatic material that presented Aschoff bodies in the myocardium into the four groups outlined above, we observed

that in the first group ("first attack" cases) myocardial Aschoff bodies are almost invariably found in the interventricular septum (T.V.*) and in the upper part of the posterior wall of the left ventricle (M.P.). The posterior wall (myocardium) of the left auricle (L.A.), left posterior papillary muscle (P.P.M.) and pulmonary conus (P.A.V.) show the presence of Aschoff bodies in about 60 per cent of the cases. The myocardial wedge between the aorta and left auricle (A.M.V.) shows Aschoff bodies in only a small percentage of the cases.

The Group 2 cases, *i. e.*, those where the individual suffered from one previous attack, show a decidedly lower incidence of auricular myocardial Aschoff bodies (approximately 20 per cent), but the same incidence of lesions in the upper part of the posterior wall of the left ventricle (M.P.). The distribution of Aschoff bodies in the other sections is similar to that in Group 1, but approximately 15 per cent lower in incidence. In Groups 3 and 4 myocardial Aschoff bodies were not found in the myocardial wedge between the aorta and left auricle (A.M.V.). They were rare in the posterior wall of the left auricle (L.A.). The distribution of Aschoff bodies in the left posterior papillary muscle (P.P.M.), the interventricular septum (T.V.) and pulmonary conus (P.A.V.) was in the same proportion as the corresponding sites in Group 1, but about 30 per cent lower in incidence. In Group 3 Aschoff bodies were found in the upper part of the posterior wall of the left ventricle (M.P.) in 90 per cent of the cases, in Group 4 in 64 per cent of the cases. Perivascular and sub-endocardial Aschoff bodies, on the other hand, in contrast to what we have termed "myocardial Aschoff bodies," seem to occur with greater frequency in Group 4 cases.

In summarizing our findings in these four groups it seems that when Aschoff bodies are found in all the standardized blocks the case almost invariably falls into the first group of our clinical classification. Furthermore, involvement of the myocardium of the posterior wall of the left auricle (L.A.) with Aschoff bodies occurs almost as frequently as it does in the posterior wall of the left ventricle (M.P.) and interventricular septum (T.V.). Conversely, the left auricular myocardium (L.A.) is seldom involved with Aschoff bodies in the remaining three groups. The incidence of Aschoff bodies in the up-

* These bracketed initials refer to the abbreviated terminology employed by Gross, Antopol and Sacks¹² to designate the standardized sections.

per part of the posterior wall of the left ventricle (M.P.) remains approximately the same (90 per cent) in the first three groups. It is somewhat lower in the fourth group. The incidence of Aschoff bodies in the interventricular septum (T.V.) is extremely high (almost 100 per cent) in the first group. It varies from 66 per cent to 88 per cent in this site in the remaining groups. This curious rearrangement in the incidence of Aschoff bodies in various parts of the heart, brought about by the clinical course of the disease, again suggests the possibility of some alteration in the reactivity of the tissues induced by the nature and frequency of previous attacks.

Quite apart from this clinical grouping of our material, it may be stated that when Aschoff bodies are present in the myocardium they will be found almost invariably either in the interventricular septum (T.V.) or posterior wall of the left ventricle (M.P.). The next most frequent sites in the order of frequency with which Aschoff bodies are found are the left posterior papillary muscle (P.P.M.), pulmonary conus (P.V.), posterior wall of the left auricle (L.A.) and myocardial wedge between the aorta and left auricle (A.M.V.).

DISCUSSION AND SUMMARY

There has been presented in this report a study of the life cycle of the myocardial Aschoff body, based on an examination of the clinical records and autopsy material from 70 cases that presented Aschoff bodies in the myocardium. It appears that these specific lesions pass through three stages in development. The earliest phases, represented by small cell coronal and reticular Aschoff bodies, have been found to occur up to the 4th week after the onset of the illness. The middle phases, represented by large cell coronal, syncytial coronal, mosaic and large irregular cell polarized Aschoff bodies, have been found to occur between the 4th and 13th week after the onset of the illness. The late phases are represented by polarized Aschoff bodies which occur from the 9th to the 16th week after the onset of the illness, and subsequently by fibrillar Aschoff bodies which occur after the 13th week of the illness.

The earliest types of specific lesions are apparently influenced in their response by the reactivity of the tissue, depending on whether there has or has not been a previous attack of rheumatic fever, and also by the state of the collagen present in the interstices between the

myocardial bundles. As a consequence, the evolution of the lesion may follow one of two main courses, determined by the initial lesion. The latter may occur in the form of the reticular or the small cell coronal Aschoff body. The final phases of the life cycle of the Aschoff body are common to both main courses.

Dividing the material into four groups representing different clinical courses, there appears to be some change both in the incidence of the types of Aschoff bodies present in the myocardium and in their localization. The findings reported here, however, can by no means be considered as furnishing sufficient statistical evidence on which to base final conclusions on this point. That the tempo of the life cycle may be considerably faster or slower than what has been described in this report seems very probable. Some of the stages in the "model" of the life cycle presented by us may be absent in some cases, abbreviated in others, or indeed, appear in the reverse order from what we have suggested. These facts can be determined with greater accuracy only after examining a much more extensive series of cases and, in the last analysis, must await confirmation by the hitherto unsuccessful transmission of this disease to animals. It is hoped, however, that further studies will be made along these lines in order that some of these interesting relations may be placed on a firmer footing.

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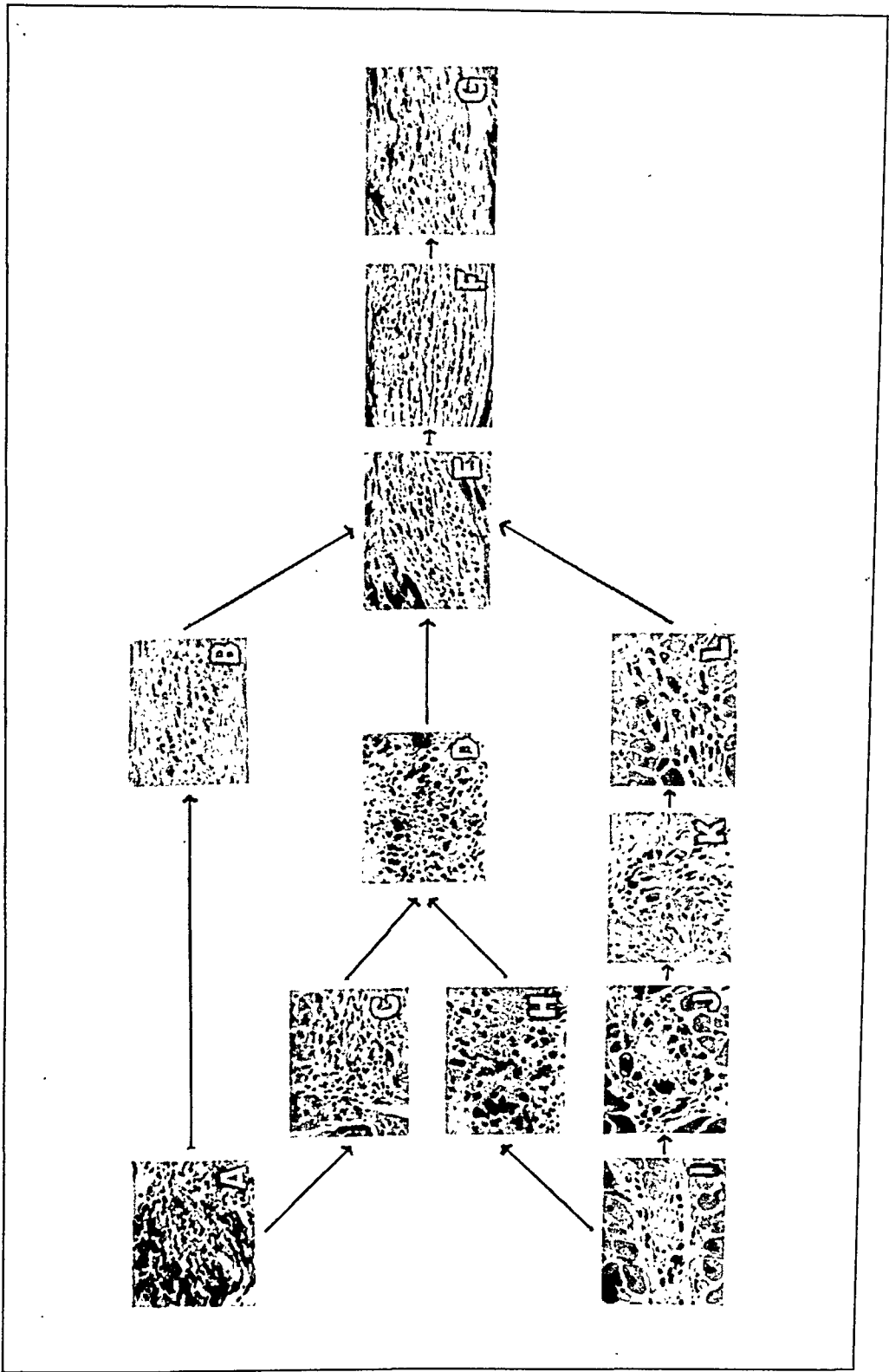
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DESCRIPTION OF PLATE

PLATE 125

FIG. 1. Aschoff body types illustrating various stages in the life cycle of the lesion. A, reticular stage; B, large irregular cell polarized stage; C, reticular stage with fusion and granular degeneration of collagen fibers; D, mosaic stage with granular degeneration of collagen; E, polarized stage showing marked spindle cell formation; F, polarized fibrillar stage; G, fibrillar stage; H, large cell coronal stage with granular degeneration of collagen; I, small cell coronal stage; J, large cell coronal stage; K, coronal mosaic stage; L, mosaic stage, compact form with beginning polarization.



THE DISAPPEARANCE OF GLOMERULI IN CHRONIC KIDNEY DISEASE *

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It is a common opinion that the ultimate fate of a glomerulus damaged beyond recovery is cicatrization and atrophy with the formation of a more or less permanent spherical hyaline scar. Experimental evidence, however, is lacking to determine whether such scars are permanent, or whether they eventually disappear. The ratio between intact and scarred glomeruli has been believed to furnish some indication of the amount of renal parenchymatous destruction. An estimate derived from such observations of the extent of renal damage would be justified if the glomerular scars were permanent, but if they were to disappear without trace the final histological picture would give less insight into the amount of damage, and even the pathogenesis of the disease, than is commonly supposed. Investigators of the pathological histology of Bright's disease have recognized tubular atrophy and disappearance since the time of Cohnheim,¹ but as indicated in a recent review by Fahr,² and in the even more recent histological studies of McGregor³ and of Oliver and Lund,⁴ the obliterated glomerulus has not been followed beyond the spherical hyaline scar. Both MacCallum⁵ and Mosenthal⁶ mention the possibility of complete glomerular disappearance. Joelson, Beck and Moritz⁷ in a study of dog kidneys at varying intervals after temporary ureteral obstruction inferred that glomeruli may completely disappear without leaving recognizable scars, providing sufficient time were allowed to elapse between injury and examination.

If the spherical hyaline scars in a diseased kidney are permanent monuments to destroyed nephrons their number plus the number of non-contracted glomeruli should equal the number of glomeruli found in a normal kidney. To investigate this the number of patent glomeruli in a kidney has been estimated by the injection method,

* Received for publication April 9, 1934.

and the proportion of injected and uninjected glomeruli and glomerular scars determined by examination of histological sections. From these observations the total number of intact and scarred glomeruli in a given kidney could be calculated.

DETERMINATION OF PATENT GLOMERULI BY INJECTION

The number of patent glomeruli was estimated by Kunkel's⁸ modification of Vimtrup's method. Human and rabbit kidneys were obtained as soon after death as possible, usually within 12 hours in the former and immediately in the latter, were weighed, and cannulae tied into the renal artery and vein. The kidney was then perfused at 140 mm. Hg. pressure with a mixture of equal parts of 2.5 per cent potassium ferrocyanide and ferric ammonium citrate after the blood had been completely washed out with physiological salt solution. It was then stripped of all fat, including that in the pelvis, weighed again and four blocks taken for histological sections. These were also weighed. By weighing the fat that had been removed the original weight of the kidney before perfusion was obtained. The kidney was cut into pieces, macerated in 50 per cent HCl for 24 to 36 hours, and then transferred to water in which the maceration was continued for 24 to 48 hours. By this time the kidney was quite soft and could easily be drawn through a tube 2 mm. inside diameter. The macerated kidney was then diluted to a suitable volume, thoroughly mixed, and the glomeruli in 2 cc. aliquots counted on a ruled Syracuse watch glass under a binocular biobjective microscope. Glomeruli are resistant to digestion and appear as deeply stained blue balls amid pieces of broken tubules. Twenty aliquots from at least three samples of the final dilution were counted.

HISTOLOGICAL EXAMINATION

The blocks taken for histological sections were fixed in acidified formalin, washed, dehydrated and embedded in paraffin in the usual manner. Sections were cut 6 microns thick at intervals of 0.1 mm. from each block and were stained either with hematoxylin and eosin or by the Van Gieson technique. The percentage of injected glomeruli, non-injected but apparently patent glomeruli, and glomerular scars was determined by differential count. Scars of

doubtful identity were counted as glomeruli, so that any error in the differential count represented more glomeruli than were actually present rather than less. This procedure gave added significance to estimated totals which were less than the expected normal. The estimation of the total glomeruli by injection and the differential counts in sections were made independently by different observers.

In making calculations it was assumed that the distribution of patent and obliterated glomeruli throughout the kidney was fairly uniform. The histological examination of blocks taken from different parts of the kidney seems to justify this assumption.

From these data two calculations were made: first, from the proportion of apparently patent but uninjected glomeruli the total number of possibly patent glomeruli was estimated; and second, from the proportion of fibrous and apparently patent glomeruli the total number of recognizable glomerular structures was calculated. There is probably an error in these estimations since a glomerulus often appeared patent but uninjected in a single section, although serial sections showed that its afferent artery was completely occluded so that it could not be injected. This, however, does not affect the comparison of the total number of glomeruli in the diseased kidney with the normal. An example of calculation follows:

Autopsy No. 4553, Jan. 11, 1934. Weight of kidney and fat before perfusion 275 gm. Weight of kidney after perfusion 226 gm. Weight of blocks for sections 2 gm. Weight of fat 110 gm. Corrected weight of kidney 165 gm. Kidney cut into small pieces and these placed in 50 per cent HCl. January 12, transferred from acid to water. January 13, diluted to 16 liters. Counts on 2 cc. samples: 174, 216, 190, 161, 176, 182, 170, 175, 166, 179, 149, 163, 160, 154. Mean 170.2 ± 12.27 . By Fisher's⁹ formula $t = 39.13$ and is significant. Estimate of injected glomeruli $170.2 \times 8 = 1361.6$ thousand. Correction for blocks taken for histological section 12 thousand. Corrected estimate of injected glomeruli 1374 thousand. Differential count of 795 glomeruli showed 98 per cent injected, 1.5 per cent apparently patent but uninjected and 0.5 per cent hyaline scars. Correcting the estimate from injected glomeruli gave 1395 thousand apparently patent, and 1402 thousand recognizable glomerular structures.

NORMAL KIDNEYS

Estimations of the number of glomeruli in the human kidney have varied tremendously — from 560 thousand to 5,700 thousand by different observers and using diverse methods. By injection of iron salts and acid digestion Vimtrup¹⁰ found 834 thousand to 1,233 thousand, Moore¹¹ 600 thousand to 1,200 thousand, Hayman and Johnston¹² 800 thousand to 1,500 thousand. The number of glomeruli in the two kidneys of the same animal have been found approximately equal (within 10 per cent) by Hayman and Starr¹³ for the rabbit and by Moore for man.

Fourteen kidneys from human subjects between 1 month and 88 years of age were injected (Table I). None had evidence of kidney disease during life, with the exception of 3 who died from acute mercuric chloride poisoning. These have been included in the normal group because of the absence of history of previous kidney disease, the lack of histological evidence of glomerular damage and the fact that a bichloride kidney may be perfused and injected as a normal one.

Of interest in this series was the observation that in a kidney from an infant 1 month of age, 10 per cent of the glomeruli were immature, non-patent structures. This confirms previous observations that postnatal maturation of glomeruli occurs in animals (cats).¹⁴ When these immature glomeruli were included in the total estimate of glomerular structures the number was equal to that found in the adult. Even in "normal" kidneys of young individuals a small number of fibrous or hyaline glomerular scars were found. This number tends to increase slightly with age, but an advanced age may be reached without any significant decrease in the total number of glomeruli. This is at variance with the observations of Moore who found that the number of glomeruli is reduced after the sixth decade. As would be expected, kidneys of very different weights may contain approximately the same number of glomeruli.

The range of the estimations of total glomerular structures in these fourteen kidneys was from 940 to 1542 thousand. The mean was $1,282.8 \pm 32.7$ thousand and the standard deviation 174 thousand. If a normal distribution be assumed in this sample of 14 kidneys (and there is nothing to indicate that the distribution is not normal), then glomerular counts differing from the mean by more than twice

the standard deviation, or below 933.7 thousand, may represent a significant reduction, while the chances are about 370 to 1 that any count differing by more than three times the standard deviation, or below 759.1 thousand, represents a true reduction. The mean of the present series differs slightly from that found by Hayman and Johnston in a series of 12 normal kidneys, but in which no correction

TABLE I
Glomerular Counts of Normal Kidneys

Autopsy No.	Sex	Age	Kidney weight	Injected glomeruli in macerated kidney	Differential count in sections				Total possibly patent	Total glomerular structures
					Number counted	Injected	Uninjected non-contracted	Fibrous		
			gm.	thousands		per cent	per cent	per cent	thousands	thousands
4480	M	1 hr.	14	1050	1520	89.0	11.0*	..	1180	1180
4495	F	1 mo.	9	944	479	90.0	10.0*	..	1049	1049
4360	F	20 yrs.	115	1004	675	92.0	7.0	1.0	1080	1091
4527	M	20 "	132	1421	781	100.0	1421	1421
4575	F	21 "	124	1242	701	99.2	0.8	..	1252	1252
4154	F	23 "	156	1230	708	96.2	1.2	2.6	1245	1279
4457	M	28 "	187	1465	548	95.0	4.8	0.2	1438	1542
4372	M	41 "	230	1300	969	99.0	0.6	0.4	1307	1312
4294	M	49 "	268	1238	974	95.5	2.0	2.5	1163	1206
4216	M	51 "	210	1530	869	99.4	0.2	0.4	1533	1539
4553	F	56 "	165	1373	795	98.0	1.5	0.5	1394	1401
A9211	M	65 "	145	905	636	96.3	0.4	3.3	909	940
4337	M	71 "	170	1023	610	85.5	0.7	13.8	1031	1196
4554	M	88 "	114	1298	739	89.0	8.0	3.0	1414	1458

* Immature non-patent glomeruli.

was made for fibrotic glomeruli. The difference in the means (1156 ± 38.8 and 1283 ± 32.6) is 127 ± 61.4 thousand, and is not statistically significant.

CHRONIC RENAL DISEASE

The abnormal kidneys have been grouped according to the type of pathological process (Table II). In these the number of obviously and possibly patent glomeruli has been recorded, as well as the total number of recognizable glomerular structures. The difference between the total possibly patent and the normal number of glomeruli indicates the reduction in the number of functioning units,

or units that may possibly be capable of any function, while the difference between the last figure and the normal measures the number of glomeruli that have disappeared so that they are no longer recognizable. Both of these estimates are probably too high, since any glomerulus that contains dye at all is counted as injected, although many of its capillaries were frequently occluded. The number of possibly patent glomeruli cannot, therefore, be taken as a measure of the extent of the filtering surface. The second figure is probably high also, because it was frequently impossible to distinguish whether an acellular hyaline scar represented an obliterated glomerulus or blood vessel.

In all groups there are instances in which there is not only a significant but even a striking reduction. This is most marked in the cases of diffuse glomerular nephritis. In the arteriosclerotic group there is a significant reduction, not only in the number of patent glomeruli, but also in the total number of recognizable glomerular structures, both in those who died of cardiac failure and in those in whom the arteriolar nephrosclerosis was an incidental autopsy finding. In two of the three patients with vascular disease who died in uremia with renal failure the renal damage had progressed rapidly. In one (4204) clinical evidence of renal impairment had been present for only a month before death, while in another (4147) it had been present less than 6 months. It is probable that in these cases death occurred before the functionless glomeruli had been hyalinized or had disappeared. In the microscopic sections from these kidneys great difficulty was frequently experienced in distinguishing patent uninjected glomeruli from non-patent but not hyalinized glomeruli, so that the count of injected glomeruli is probably a closer estimate of the number of possible functioning structures than the corrected estimate.

It would appear from these data that more than half of all the glomeruli in a kidney damaged by vascular disease or chronic inflammation may disappear without leaving recognizable scars. Furthermore, the disappearance of renal parenchyma, as indicated by the number of remaining glomeruli and glomerular scars, need not be paralleled by a corresponding reduction from the expected normal weight of the kidney.

TABLE II

Glomerular Counts of Pathological Kidneys

Autopsy No.	Sex	Age yrs.	Kidney weight gm.	Injected glomeruli in macerated kidney thousands	Differential count in sections				Total possibly patent thousands	Total glomerular structures thousands	Pathological diagnosis
					Number counted	Injected per cent	Uninjected non-contracted* per cent	Fibrous† per cent			
4147	M	41	150	220	623	37	8	55	269	595	Arteriolar nephrosclerosis Death due to renal insufficiency
4204	F	48	94	543	612	60	10	30	634	905	
4100	M	44	100	222	1015	32	31	37	437	693	
4359	M	41	212	705	993	66	19	15	908	1068	Death due to cardiac failure
4165	M	85	97	467	1123	83	11	6	529	563	
4152	F	38	109	241	808	42	45	13	499	574	
4145	M	67	140	330	1017	60	9	31	378	550	
A9127	F	67	141	734	549	89	1	10	740	824	
4530	F	45	111	386	741	42	20	38	570	919	Death due to cerebral hemorrhage
4172	F	67	83	391	531	93	2	5	399	420	Incidental autopsy finding
4563	F	72	109	663	526	83	4	13	695	799	
A9160	M	46	63	430	927	93	1	6	434	462	
4173	F	52	135	619	720	97	1	2	625	638	
4319	F	49	156	301	508	57	21	22	412	528	Chronic diffuse glomerular nephritis
4274	F	27	72	132	441	43	12	45	169	307	
4557	F	55	39	93	432	35	3	62	101	266	
A9167	F	43	34	127	762	82	3	15	132	155	
4137	F	60	130	771	756	96	2	2	787	803	Chronic pyelonephritis
4139	M	75	170	850	784	95	1	4	859	895	
4485	M	55	118	485	793	95	3	2	501	511	
4200	M	21	163	1119	1086	97	2	1	1142	1154	Focal glomerular nephritis

* It was frequently impossible to determine whether non-contracted, non-dye-containing glomeruli were potentially patent or not.

† It was frequently impossible to determine whether an acellular, hyaline scar represented an obliterated glomerulus or blood vessel. Such scars were counted as glomeruli and probably exaggerate the estimated total number of glomeruli.

THE MORPHOLOGICAL ASPECTS OF GLOMERULAR DISAPPEARANCE

If this evidence be accepted as indicating that glomeruli disappear without trace, the histogenesis of this process demands study. For this purpose two uninterrupted series of 200 sections, each 6 microns in thickness, were cut; one from a kidney the seat of nephrosclerosis and the other from a kidney the seat of chronic diffuse glomerulonephritis. The sections from these two series were stained with hematoxylin and eosin, Masson's trichrome light green, Mallory-Heidenhain azan carmine, and Foot's silver carbonate, in rotation, so that the various structural characteristics of a given glomerulus could be observed in adjacent sections. In a number of instances it seemed advisable to study the various elements of a glomerulus in the same section. To do this a glomerulus was photographed repeatedly at a constant magnification, destaining and staining by a different technique between each photograph. In addition to the stains mentioned above, Mallory's phosphotungstic acid hematoxylin was found especially useful for the identification of collagen and fibroglia. Glomerular scars in kidneys the seat of chronic pyelonephritis and hydronephrosis were also studied in this manner.

Normal and diseased glomeruli have recently been described in detail by McGregor, who did not, however, study the components of the glomerular scar. This spherical hyaline body, when stained with hematoxylin and eosin or azan carmine, is apparently of too simple a structure to justify further investigation. Regardless of whether the glomerular damage was the result of arteriolar nephrosclerosis, chronic diffuse glomerular nephritis, chronic pyelonephritis or hydronephrosis, the final spherical hyaline glomerular scars are for the most part indistinguishable from one another. Even after the glomerulus has been converted into a structure which in hematoxylin and eosin preparations appears to be a homogeneous, acidophilic sphere, special stains (azan carmine, phosphotungstic acid hematoxylin) may disclose the shadowy outlines of the wrinkled, thickened basement membrane of arteriolar nephrosclerosis (Fig. 1 B) or the more fibrillar peripheral remnant of a capsular crescent denoting inflammatory change. These distinguishing features are subsequently lost with complete hyalinization (Fig. 2 B).

Four fibrillar elements are concerned in the organization and disappearance of the glomerular scar in chronic renal disease. The only stainable intercellular structure normally present in a glomerulus is the basement membrane of the tuft. This membrane is covered by epithelium and lined by endothelium and is in structural continuity with the capsular and tubular membranes (Fig. 4 A). Furthermore, it stains in the same manner except that it is not argentophilic, whereas the membrane surrounding capsule and tubule has an argentophilic component (Fig. 4 B). In nephrosclerosis the argentophobic glomerular and the argentophobic portions of capsular and tubular basement membranes are thickened, but the argentophilic component is not altered, the accretion of substance being inside of it (Figs. 1 B and 1 C). With contraction and hyalinization of the glomerular scar the more or less continuous argentophilic capsular membrane becomes disrupted and there is irregular prolongation of the interstitial silver staining fibrils into the periphery of the hyaline mass (Fig. 2 C). Argentophilic fibrils also appear at the vascular hilum and develop in the center of the scar. The original argentophobic tuft membrane has in the meantime lost its identity and has become incorporated in the hyaline mass. The scar gradually loses its spherical contour, is rendered irregular by superficial concave defects, and within it there are focal areas of rarefaction (Fig. 3 B). The penetrating peripheral and central argentophilic fibers become confluent and conform in pattern to that of the interstitial tissue of the kidney, and the last recognizable trace of the glomerulus is a local collection of small, irregularly outlined, confluent hyaline bodies lying in the interstices of an argentophilic mesh.

The other two fibrillar elements are collagen and fibroglia. Collagen fibrils appear only in the peripheral portion of the glomerular scar and are not seen in the more advanced examples of organization. Their situation would suggest that they are derived from the capsular fibrous tissue. Fibroglial processes develop within the substance of the hyaline sphere and do not appear to play any important part in its organization. The probable source of these processes is from capillary endothelial cells that survive within the scar. These cells enlarge, elongate, and come to resemble fibroblasts. No example of a completely acellular scar was encountered and no evidence of fibroblastic penetration of the scar was recognized.

Fatty degeneration of the scar occurs especially in kidneys the

seat of rapidly progressive disease. The degeneration is frequently severe enough to leave large defects in the otherwise hyaline mass. In the less rapidly progressive types of renal disease fatty degeneration is inconspicuous. Calcification is occasionally present in the form of finely dispersed granular deposits, but is rarely extensive.

THE DISAPPEARANCE OF GLOMERULI FOLLOWING EXPERIMENTAL RENAL INJURY IN RABBITS

Various attempts were made to produce unilateral renal injury of sufficient severity to effect a certain amount of diffuse irreparable glomerular damage, so that the total number of surviving glomerular

TABLE III
Glomerular Counts of Rabbits' Kidneys

Rabbit No.	Left (control) kidney				Right (injured) kidney				Type injury	Recovery period
	Injected glomeruli in macerated kidney	Differential count		Total glomerular structures	Injected glomeruli in macerated kidney	Differential count		Total glomerular structures		
		Injected	Uninjected and scars			Injected	Uninjected and scars			
	<i>thous'ds</i>	<i>per cent</i>	<i>per cent</i>	<i>thous'ds</i>	<i>thous'ds</i>	<i>per cent</i>	<i>per cent</i>	<i>thous'ds</i>		<i>days</i>
101	167	98	2	170	137	98	2	140	X-ray	27
102	144	97	3	149	115	89	11	130		63
131	163	99	1	165	146	98	2	149		71
133	181	98	2	185	122	94	6	130		71
134	183	99	1	185	126	88	12	143		71
132	179	99	1	181	130	99	1	132		77
130	140	97	3	144	33	88	12	39		153
143	116	100	..	116	89	90	10	99	Ureter kinked	56
144	99	98	2	101	36	99	1	37		56
145	166	98	2	169	96	94	6	102		56
2842	128	100	..	128	120	98	2	123	Vein clamped 17 hrs.	90
148	178	100	..	178	179	100	..	179	Control	

structures could be compared with the number of glomeruli in the other (normal) kidney. Of the methods tried, temporary partial ureteral obstruction and exposure to X-ray proved most satisfactory in our hands. For unilateral injury by X-ray the kidney was delivered through a lumbar incision, the animal protected with lead foil,

and the exposed kidney subjected to from 2 to 5 human erythema doses. Partial ureteral obstruction was produced by kinking the ureter just above the bladder by suturing in to the latter. After 35 days the animal's abdomen was again opened, the sutures cut and the ureter straightened. At this operation the ureter was found to be dilated, while at autopsy this had disappeared.

As indicated in Table III, complete disappearance of as many as 70 per cent of all the glomeruli in a rabbit's kidney may occur without the persistence of scars to denote their previous existence. This lends confirmation to the suggestion previously made by Joelson, Beck and Moritz regarding glomerular disappearance in the dog.

DISCUSSION

It has been shown that both in chronic renal disease in man and in experimentally produced glomerular injury in rabbits a large proportion of the glomeruli in a given kidney may disappear, leaving no recognizable trace. In the case of the rabbits, where the disease was not progressive, there was not even any condensation of the interstitial connective tissue to indicate the loss of parenchyma. In man the chronic progressive nature of the disease made interstitial fibrosis a constant finding, even though the recognizable glomerular scars were not numerous enough to account for more than a fraction of the obliterated glomeruli. The reduction of the number of glomeruli was not paralleled by a corresponding reduction from the expected normal weight of the kidney.

If so large a proportion of the glomeruli in chronic renal disease can disappear without trace, as is indicated in Table II, the final histological examination of the kidney may give less information concerning the pathogenesis and severity of the disease than is commonly thought. If a kidney, having originally an expected normal number of about one million glomeruli, can lose as many as three-fourths of these without leaving recognizable scars of those lost, it is not fair to assume that the changes affecting the one-fourth remaining were necessarily the same as those that occurred in the glomeruli that have disappeared. The final pathological diagnosis of the kidney is frequently made on a basis of the preponderant change seen. This may involve a weighing of the evidence of arteriolar sclerosis against the evidence of inflammation. If complete

glomerular disappearance occurs to the extent indicated in this investigation the final pathological picture may throw but little light on the pathogenesis of certain types of chronic renal disease.

SUMMARY AND CONCLUSIONS

1. The number of possibly patent glomeruli and glomerular scars has been estimated by a combination of injection and histological methods.

2. The average number of glomeruli in 14 normal human kidneys was $1,282.8 \pm 32.7$ thousand.

3. In chronic renal disease not only the number of patent glomeruli but the total number of recognizable glomerular structures was reduced. This was most marked in chronic glomerular nephritis. The number of possibly patent glomeruli frequently falls below 500 thousand and may be below 200 thousand. The total number of recognizable glomerular structures, including scars, was frequently below 600 thousand and in some instances below 300 thousand.

4. Since large numbers of glomeruli may disappear during the course of chronic renal disease it is suggested that the final histological pattern may not give as much information concerning the pathogenesis or severity of the disease as is commonly thought.

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DESCRIPTION OF PLATES

PLATE 126

FIG. 1 A, B and C. Three photographs of the same section including a glomerular scar in an early stage of organization.

A, stained by hematoxylin and eosin.

B, stained by the Mallory-Heidenhain azan carmine method.

C, stained by Foot's silver carbonate method. $\times 300$.

In A the spherical hyaline scar appears quite homogeneous. In B the denser staining, thickened basement membrane of the tuft can be identified. In C the argentophilic capsular membrane is quite intact, but in the center of the scar reticulum fibrils, continuous in serial sections with the vascular hilum, may be seen.

FIG. 2 A, B and C. Three photographs of the same section including a glomerular scar which shows more advanced organization than is seen in Fig. 1.

A, stained by hematoxylin and eosin.

B, stained by the Mallory-Heidenhain azan carmine method.

C, stained by Foot's silver carbonate method.

In both A and B the scar appears homogeneous but in C the capsular reticulum is discontinuous and the scar shows peripheral penetration from the interstitial tissue and central penetration from the vascular hilum by argentophilic fibrils. $\times 300$.



IA

IB

IC



PLATE 127

FIG. 3 A and B. Two photographs of the same section cut through the middle of the almost completely absorbed glomerular scar.

A, stained by hematoxylin and eosin.

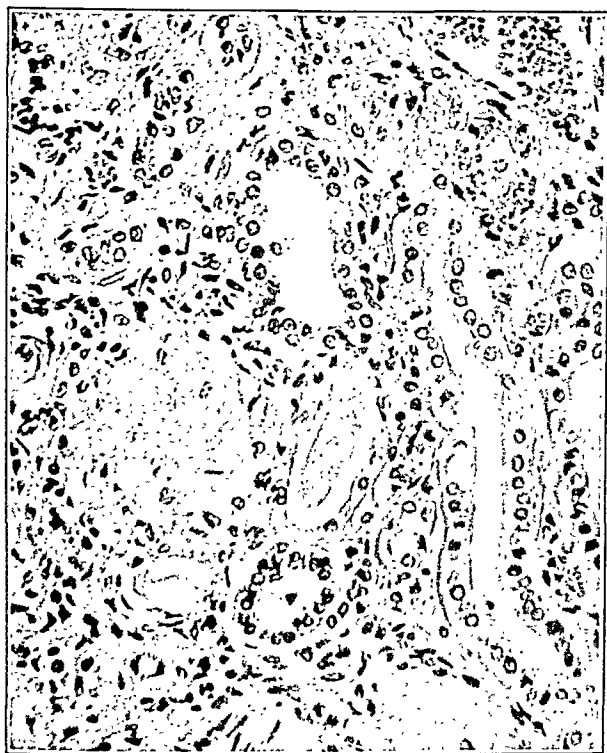
B, stained by Mallory-Heidenhain azan carmine method.

In A the scar appears to be homogeneous and spherical. In B the scar is seen to be irregularly absorbed from within and from without. A comparison of the distribution of nuclei in A with the distribution of the areas of hyaline absorption in B indicates that the disappearance of hyaline occurs around cells that appear to be fibroblasts. $\times 385$.

FIG. 4 A and B. Two photographs of normal glomeruli.

A, glomerulus stained by the Mallory-Heidenhain azan carmine method to show the continuity of the capsule (and tubular) basement membrane with that of the tuft. $\times 385$.

B, glomerulus stained by Foot's silver carbonate method to show that the argentophilic component of the capsular and tubular membrane is not continued into the basement membrane of the tuft. $\times 385$.



3A



4A



3B



4B

ACTINOMYCOSIS OF TUBES AND OVARIES *

REPORT OF A CASE

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The table and bibliography appended hereto have been made as comprehensive as possible in an attempt to collect in the English literature all cases of actinomycosis of the internal female genitalia. There have been found 71 published cases,† some of which are listed as parametrial and perhaps should not be included. The American literature records 7 cases, the English 6 and the remainder are in other languages. Of the 71 cases 45 died, in 7 the outcome is not recorded, 8 were improved and only 11 cures are claimed or inferred. Of the 11 cures only 2, those of Martin and Martius, are reported as exceeding 1 year. Both patients were well, over 3 years after discharge. It has been learned in a personal communication that Brickner's patient, who had been well for 2 years when reported in 1924, died in 1930 of actinomycosis.

Helwig, Brickner, and Draper and Studdiford give good reviews of relatively recent date in English, and Nürnberger's chapter on the subject is excellent in German. These articles give reviews of current opinions as to etiology, diagnosis and treatment. The intestinal tract is possibly the primary site of infection, and extension is generally by continuity of tissue. Early operative intervention, potassium iodide, Roentgen therapy and possibly yatren appear to be the treatments of choice. These facts appear generally agreed upon.

* Received for publication November 27, 1933.

† One additional case has been indexed since submitting the above article for publication. Rumpf, E. Geschlossene Ovarialaktinomykose. *Zentralbl.f. Gynäk.*, 1933, 57, 1216-1218. This case occurred in a female 40 years of age, whose appendix was removed in May, 1929, at which time the internal genitalia appeared only slightly injected. In September of that year she was taken suddenly ill, a mass was found in the left pelvis and this grew to the navel. Puncture drainage by vagina was followed by temporary improvement. In May, 1930, severe symptoms returned and laparotomy was performed July 21, 1930. Bilateral ovarian and parametrial involvement by actinomycotic abscesses was found. Death occurred 5 days after operation from peritonitis.

TABLE I
Published Cases of Actinomyces of Internal Female Genitalia

No.	Author	Date of publication	Age yrs.	Location	Treatment	Duration	Result
1	Zemann ¹	1883	30	Parametrium	Not given	17 mos.	Died
2	Zemann ¹	1883	40	Right tube	Not given	1½ mos.	Died
3	Zemann ¹	1883	50	Left ovary	Not given	6 mos.	Died
4	Middeldorpf	1884	32	Ovary	Symptomatic	15 mos.	Died
5	Illich	1892	?	Parametrium	Operation	4 mos.	Died
6	Santer ²	1892	36	Parametrium	Operation	4 mos.	Died
7	Santer ²	1892	35	Parametrium	Operations	15 mos.	Died ³
8	Santer ²	1892	26	Parametrium	Operations (drainage)	3½ yrs.	Died
9	Redtenbacher	1893	19	Left ovary	Operation (drainage)	14 mos.	Died
10	Stewart and Muir	1893	35	Uterus and adnexa	Not given	10½ mos.	Died
11	Giordano	1895	64	Ovaries and right tube	Operation and zinc chloride	4 yrs.	Cured (?)
12	Habel	1896	45	Uterus	Puncture (drainage)	5 wks. (?) ⁴	Died
13	Litten	1900	39	Right ovary	Not given	1 yr.	Died
14	Fehmers	1901	?	Right ovary	Operation (dr.) and KI	2½ yrs.	Died
15	Fehmers	1901	?	Parametrium	Operation (dr.) and KI	8 mos.	Died
16	Hart	1902	49	Parametrium and left ovary	Operation	5½ mos.	Died
17	Henriot	1902	21	Left adnexa	Operation	17 mos.	?
18	Geldner	1903	15	Ovaries	Operation	1 yr.	Died
19	Rosenstein	1904	17	Right ovary	Operation and KI	4½ yrs.	Cured (10 mos.)
20	Verocay	1905	14	Uterus and adnexa	Symptomatic	5 mos.	Died
21	Zwintz	1905	41	Parametrium and adnexa	Puncture (dr.) and iodine	18 mos.	Died
22	Litten and Levy	1906	44	Tubes	Symptomatic	?	Died
23	Schlagenhauser	1906	38	Right ovary	Operation (dr.) and KI	22 mos.	Died
24	Schlagenhauser	1906	39	Right tube and left ovary	Operation (drainage)	9½ mos.	Died
25	Hamm	1906	39	Tubes	Operation	13 yrs. (?)	?
26	Martin	1906	?	Parametrium	Operation (dr.) and iodine	2½ yrs.	Cured (?) ⁵
27	Neulhauser	1907	60	Uterus and right ovary	Operation	19 mos.	Died
28	Guicciardi	1907	18	Parametrium	Operation (drainage)	3 mos.	Improved
29	Thompson	1907	?	Left ovary	Operation	2 yrs.	Died
30	McMorrow	1908	?	Parametrium	Operation (drainage)	3½ mos.	Died
31	Hamm and Keller	1909	34	Uterus and adnexa	Operation	6½ yrs.	Died
32	Taylor and Fisher	1909	34	Right ovary	Operation and KI	4½ yrs.	Improved
33	Leith	1909	?	Ovary and tube	?	?	?
34	Bondy	1910	28	Right and left adnexa	Operation (dr.) and KI	11 mos.	Died
35	Wagner	1910	19	Right and left adnexa	Operation, KI, copper ⁶	7 yrs.	Cured (?)
36	Shaw	1910	38	Parametrium and left ovary	Operation ⁷	11 mos.	Died

The following case is reported chiefly because of the cure in 4 years. The history is rather long but interesting and hence included somewhat in detail; it would indicate approximately 10 years duration of infection prior to operation.

REPORT OF CASE

J. A. B., a white, female, aged 31 years, was admitted to the Letterman General Hospital, May 3, 1929.

Previous History: The patient was born in European Russia in 1897 and lived at her birthplace until 1917. She was married in 1913, separated from her husband during the World War (1914-1918) and divorced in 1918 without resuming marital relations. Between 1913 and 1919 she had several attacks of "malaria," the diagnosis being clinical only, but the attacks were relieved by quinine. She also had frequent "colds" as a schoolgirl, each of few days duration. She has been more or less constipated all her life. In 1918 she went to Omsk, Siberia, and in 1919 to Vladivostok, where she remained until 1922. In 1919 at Vladivostok she spent 35 days in the City Hospital with severe pain in the right lower quadrant of the abdomen, being treated by vaginal douches and ice externally. In 1919 she met her present husband and has accompanied him since. From 1922 to 1923 she was in Shanghai, suffering from occasional pains in the right lower quadrant, these being at times sharp, as if pulling. They had no relation to menses. From 1923 to 1925 was spent in the Philippine Islands. According to her statement she was twice in the Sternberg General Hospital, Manila, P. I.

History from Sternberg General Hospital: Admitted Aug. 9, 1924, with a history of "ovarian trouble" for 3 years, severe hypochondriac pain for 2 months and acute exacerbation the day prior to admission. No nausea, vomiting or constipation. Weight 148 pounds (normal). Tenderness over McBurney's point. Appendectomy August 10th. The appendix was closely adherent and posterior to the cecum. Urine showed a few pus cells. Feces showed segments of tapeworm. Blood: leukocytes 6800 with 64 per cent neutrophilic polymorphonuclears on August 11th. Wassermann negative. The patient was up in a wheelchair August 22nd (12 days postoperative) and was discharged from the hospital August 31st.

Vaginal smear, on October 3rd was reported positive for pus and Gram-negative intracellular and extracellular diplococci.

Readmitted to Sternberg General Hospital Jan. 6, 1925. This history records dengue fever in July, 1924, some dysmenorrhea and leukorrhea, tapeworm, and pain in region of right ovary. Weight 155 pounds. Tenderness present in both ovarian regions; no masses palpable. Tenia solium removed by vermifuge. Cyst of right Bartholin's gland incised January 16th. Cervical discharge contained pus and a few Gram-positive bacilli. Urine and blood negative. Discharge from cyst of Bartholin's gland contained pus and intracellular Gram-negative diplococci. Cervical smear on January 17th showed no pus but endothelial cells with included bacteria. Operative scar reported non-adherent. Discharged Jan. 22, 1925. (No histological examination of appendix recorded; inquiry in 1929 failed to discover any retained tissue.)

Letterman General Hospital History: The patient stated that the abscess of the labium had existed for 5 years and dates the vaginal discharge as beginning after appendectomy in 1924. Continual use of a pad was required, being renewed daily. She also stated that the pain in the right lower quadrant was much worse after the appendectomy. In 1925 she started for the United States on a naval transport but while at Shanghai went to a Polish doctor who said she should have immediate treatment to prevent cancer. For 3 months he treated her by daily applications to or into the cervix, followed by tamponage, with some dark medicament, and daily douches before each visit to his office; menstruation stopped during this treatment for a period of 4 months. From Shanghai she came to San Francisco, where she has resided since except for 3 or 4 months spent in San Diego in 1925. In San Francisco she was at first treated three times a week by a private physician who used tamponage and douches. There was no improvement or change in the pain during either of these courses of treatment. Then followed treatment for 6 months at a San Francisco hospital, where X-ray was used for diagnosis and the patient informed that her trouble was due to adhesions. She was given a diet and oral medication, losing about 36 pounds during these 6 months (168-132). She was then sent to Letterman General Hospital by a naval surgeon.

Obstetrical History: Menses began at 15 years, were regular and of the 21 day type, 4 to 5 days heavy flow but no pain. The only interruptions have been in 1914, when she became pregnant but miscarried at 3½ months; in 1920, when she again miscarried at 3 months; and in 1925 as recorded. The patient emphatically denies any intervention during either pregnancy.

Present Illness: Admitted May 3, 1929, as an emergency case. She had been vomiting green material for 18 hours, according to her husband's statement, with severe pain across the lower abdomen. She had had a small bowel movement the day of admission and one the previous day. There was always tenderness in the suprapubic region and she had had numerous previous attacks of pain, but none so severe. The pain was chiefly across the lower abdomen, with epigastric distress.

Physical Examination: Pulse 102, temperature 99.2° F, respirations 24. Pulse regular and of good quality. General condition fair. Blood pressure 110/80. Positive findings: moderate leukorrhea; cervix elongated, especially the anterior lip; uterine fundus enlarged and irregular on posterior surface; moderate resistance in both adnexa but examination unsatisfactory because of pain on pressure upward from vagina or upon abdominal pressure; abdomen moderately distended; liver not enlarged; spleen not palpable; marked tenderness over lower abdomen, especially in suprapubic region; no definite muscle rigidity.

Progress and Laboratory Findings:

May 3rd: White blood cell count 15,850, polymorphonuclears 83 per cent at 4:30 P.M. Passed gas and had bowel movement. Urine showed trace of albumin, many pus and epithelial cells. Feces showed undigested food.

May 4th: White blood cell count 8100, polymorphonuclears 65 per cent.

May 5th: White blood cell count 4900, polymorphonuclears 68 per cent. Urine showed a few pus and blood cells.

May 6th: Blood Wassermann negative.

May 7th: On pelvic examination the external genitalia were negative, but there was a slight leukorrheal discharge in the vagina. The cervical canal was patulous with a plug of stringy mucus present; no erosions or cysts were noted;

fibrosis of the anterior lip was more marked at the neck of the cervix. The uterus was hard with irregularities on the anterior surface and both sides. On the left posterior surface at the junction of the neck and lower uterine segment there was an indefinite mass. Adnexa: masses were felt at both cornua suggestive of bilateral salpingitis; the ovary on the left was not definitely made out but seemed distinct from the mass at the cornu and apparently was prolapsed and slightly enlarged; what seemed to be a definite cystic ovary was felt on the right side.

May 13th: Operation; (1) release of intestinal obstruction, (2) oophorectomy and salpingectomy, bilateral. Paramidline incision, pubis to umbilicus. The upper abdomen was apparently negative, the lower abdomen a mass of adhesions. The uterus and adnexa were hidden by adherent intestinal loops. The bladder wall was much inflamed. At the left cornu of the uterus the bladder, tube and an epiploic appendage of sigmoid were involved in a suppurating area which might have been a diverticulum of the bladder; however, separation showed the bladder wall apparently intact and the raw area was covered by peritoneum. Both tubes and ovaries were found oozing pus. It was not possible to save any portion of either ovary so both tubes and ovaries were removed. There were thick, indurated masses involving the sigmoid and ileum, all matted together with these suppurative tubes and ovaries. Many areas looked malignant but the condition was considered probably entirely inflammatory. Upon completion the pelvis was dry and the intestine patent throughout. Raw areas were reperitonealized and the sigmoid arranged behind the uterus to prevent entry of the small intestines into the pouch of Douglas. Closure in layers. The patient's condition was good on return to the ward.

June 3rd: X-ray showed the chest to be negative for pulmonary pathology.

June 9th: Discharged, the wound having healed by first intention.

PATHOLOGIST'S REPORT

Gross Examination: The specimen, as received in 10 per cent formalin, consisted of one tube with an ovary attached and another tube and two separate (ovarian?) tumor masses. The tubes were thick-walled, congested and edematous. Both ovaries showed large, pale tumor masses considered probably carcinoma, at first. Later examination showed these to consist of multiple yellowish lobules, partially encapsulated and partially confluent, with strands of dense white tissue between the lobules and numerous small ragged foci in the centers of the cut surfaces of these lobules. Some similar foci were seen in the walls of both tubes. In the ovary adherent to the tube a large corpus luteum was noted.

Microscopic Examination: Multiple abscesses are seen in the ovaries and tubes, with granulomatous walls. There is no evidence of malignancy. The ovaries are chiefly involved. Although no

actual granules can be seen in a limited number of sections of tube, however, there are multiple abscesses in the walls, pus in the lumens, granulomatous walls about the abscesses and the inflammatory picture is identical with that seen in the ovary. One large granule is seen at the edge of the ovary very close to the tubo-ovarian adhesion. The smaller fungus granules are composed of centers that are not remarkable when stained with hematoxylin-

TABLE II

Other Cases Published or Referred to in the Literature, Not Included in Table I

No.	Author	Date of publication	Reason for omission from table
1	Bostroem	1890	Apparently same case as Middeldorpf's No. 4
2	Regnier	1894	Genitals not stated involved
3	Lieblein	1900	Skin of external genitalia involved only
4	Bongartz	1902	Vulva involved only
5	Trapl	1913	Right labium involved only
6	Reifferscheid	1924	Cannot locate with reference given
7	Richter	1927	Does not specify involvement of female genitalia

eosin but show innumerable, intertwining Gram-positive hyphae when stained by MacCallum's method for demonstrating Gram-positive and Gram-negative organisms. At the periphery of these granules the threads extend between adherent pus cells and can be seen in irregular broken form, with coccoid and bacilliform portions. Some of the small separate fragments are diphtheroid in appearance. In some sections the peripheral clubbed eosinophilic encapsulation of these hyphae is well demonstrated but in the larger clubs no filaments can be seen. One additional notation is made, not found described elsewhere. This is the large crystalline structure of the centers of the older colonies, these staining with eosin, being somewhat radial in distribution and having branched, spike-like peripheries. Such structure is seen in sections of the same granules with both stains and occupies most of the granule, only the periphery presenting many filaments. These were considered deposits of unknown composition.

Diagnosis: Actinomycosis of ovaries and tubes.

SUBSEQUENT HISTORY *

The patient has been carefully followed and has remained in excellent health since discharge from the hospital in 1929. She was last reported on April 25, 1933, at which time she weighed 155 pounds, stated that she was not constipated and on careful pelvic examination no tenderness, masses, induration or fixation could be detected.

SUMMARY

1. Seventy-one published cases of actinomycosis of the internal female genitalia are listed.
2. Of these 45 individuals died, 8 were improved, in 7 the outcome is doubtful, and only 11 are possible cures.
3. The case reported showed involvement of both tubes and ovaries. The patient was operated upon and treated by potassium iodide, and is well 4 years after operation.
4. Tabulation of some features of published cases is presented.

NOTE: It is desired to express appreciation of the courtesy and cooperation of the following officers: Lt. Colonel F. S. Wright, M.C., who was Chief of the Surgical Service at Letterman General Hospital and who performed the salpingo-oophorectomy, Major H. S. Villars, M.C., who was Ward Surgeon at that time, and Colonel R. F. Metcalfe, M.C., at present Chief of the Surgical Service at Letterman General Hospital and who has kindly examined the patient several times for the author.

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* Since this article was submitted for publication, subsequent examination of the patient on April 17, 1934, showed her to be in excellent health; her weight was 153 pounds and there was no evidence of pelvic or abdominal masses. Hence this case has remained well for 5 years after operation, with no evidence of recurrence.

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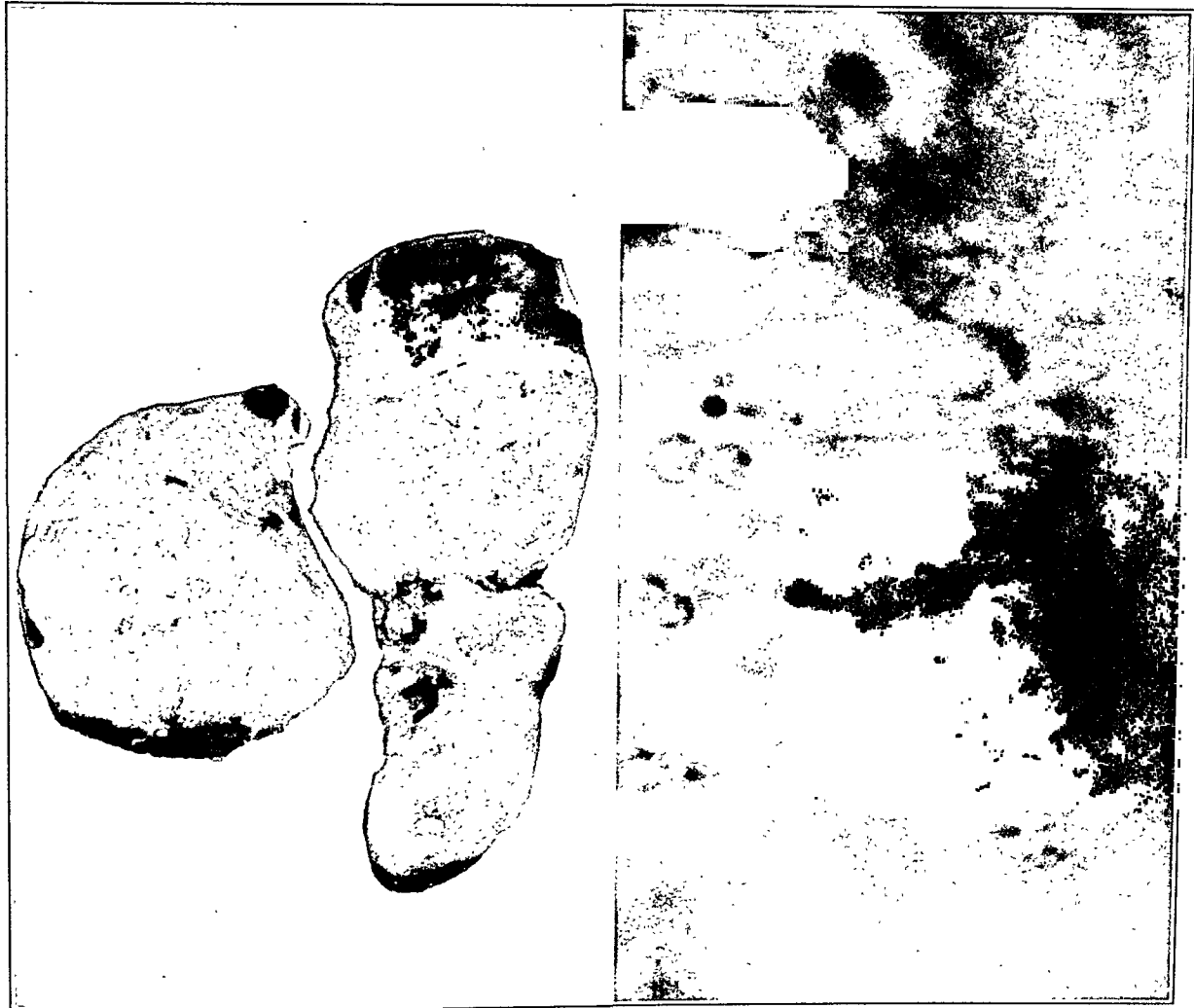
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DESCRIPTION OF PLATES

PLATE 128

- FIG. 1. Ovarian and tubo-ovarian masses in gross. Note the fibrosis, alveolar arrangement and minute central foci of necrosis. Natural size. (Army Medical Museum Neg. No. 49885.)
- FIG. 2. Higher magnification of upper edge of granule seen in Fig. 3, showing hyphae in more detail and extending into the clubs. MacCallum's stain. $\times 2000$. (Army Medical Museum Neg. No. 49884.)
- FIG. 3. Fungus granule showing mycelial maze of hyphae; hyphae extending into clubs and cellular reaction, chiefly polymorphonuclears. MacCallum's stain. $\times 810$. (Army Medical Museum Neg. No. 49883.)
- FIG. 4. Edge of granule showing higher power view of clubs. Hematoxylin and eosin stain. $\times 2000$. (Army Medical Museum Neg. No. 31018.)



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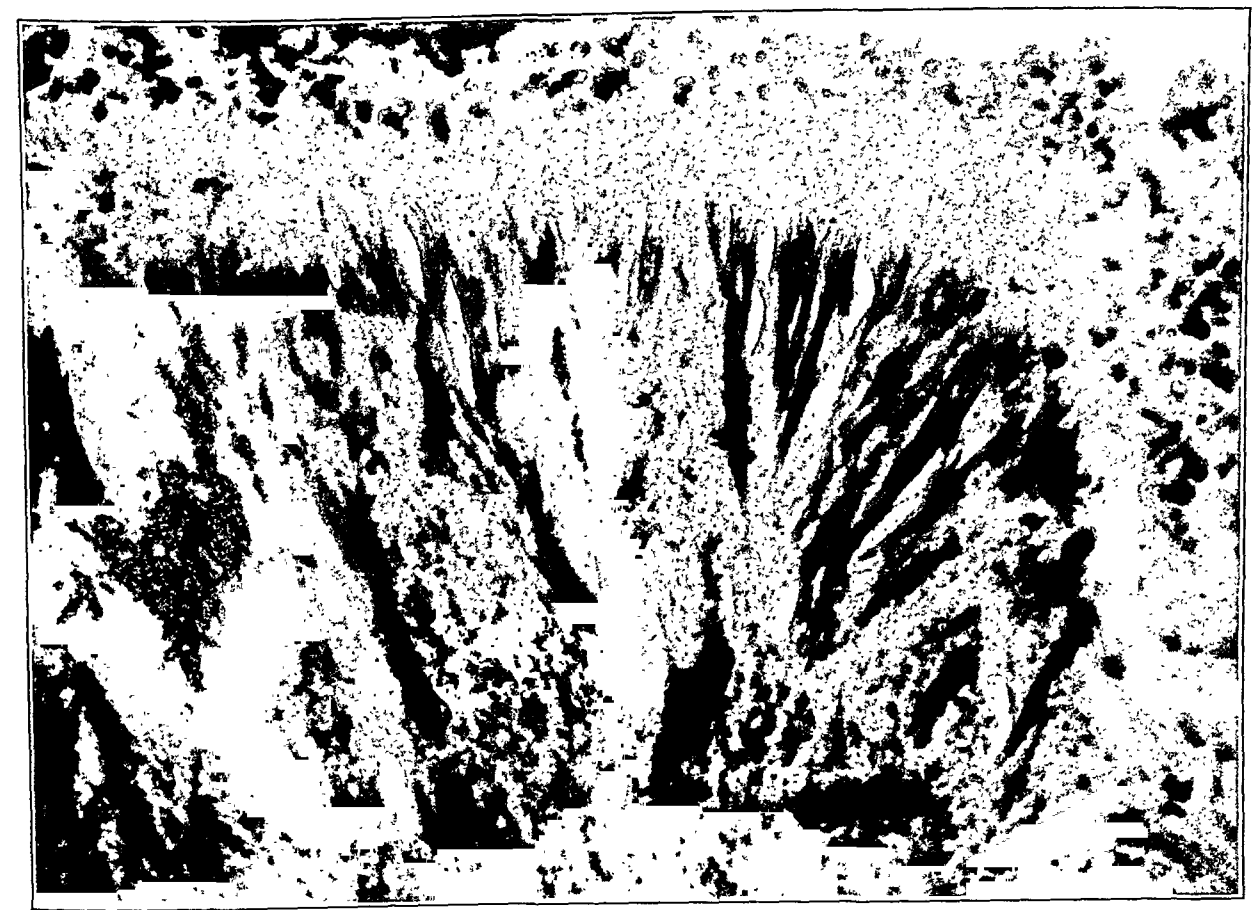
PLATE 129

FIG. 5. This granule is mostly composed of the crystalline deposit, unidentified, not found previously described. About the periphery hyphae still exist. Note purulent wall about granule. Hematoxylin and eosin stain. $\times 91$. (Army Medical Museum Neg. No. 48172.)

FIG. 6. Higher magnification of left edge of granule in Fig. 5, showing crystalline deposit in greater detail. The zone between this and the cellular exudate is a mycelial mass. Hematoxylin and eosin stain. $\times 515$. (Army Medical Museum Neg. No. 48171.)



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6

EXTRAGENITAL CHORIONEPITHELIOMA IN A MALE *

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The existence of a primary extragenital chorionepithelioma in the male has been questioned by Prym and by Oberndorfer in their recent writings. Both declare that extragenital chorionepithelioma in the male, when present, is always consequent upon a primary tumor in the testis. The case here reported, however, appears to fulfill all the requirements necessary to establish the primary extragenital origin of such a tumor.

REPORT OF CASE

Clinical History: No. 7813. B. L., a reporter, aged 22 years, was admitted to the medical service of the Mount Sinai Hospital with a harassing cough productive of thick mucoid sputum for 15 months. A postnasal drip was present at the onset. A tonsillectomy had been done with no symptomatic relief. For 13 months the cough became progressively worse and the sputum increased in amount. Two months ago the sputum became blood-streaked and pain appeared in the lower anterior chest. Two weeks prior to his admission to the hospital he awoke feeling chilly. He coughed up blood which, he was certain, came from his lungs. An obstinate anorexia, nausea and vomiting occurred with attempts to eat. A progressive dyspnea and orthopnea appeared. He lost 25 pounds in weight in the 2 weeks. His temperature rose slightly 1 day before admission.

Physical examination revealed a young man, well nourished, cyanotic and dyspneic (respirations 40). Attacks of hacking cough and vomiting were precipitated by movements in bed. There was a slight right exophthalmos. There was no tracheal deviation or fixation. The veins of the neck were engorged. The thyroid was normally palpable. The chest showed moderate emphysema. There was slight dullness and diminished fremitus throughout. There were occasional inspiratory crepitant râles present at the angle of the right scapula and anteriorly below the nipple. The heart was negative. Irregularly distributed tenderness was present throughout the entire abdomen. The right testis was small and firm; the left was slightly larger. The prostate was enlarged, but not tender or firm. There were irregularly distributed sites of tenderness over the sternum, right lower costal margin extending upward to the nipple, and both humeri.

* Received for publication January 8, 1934.

Presented at the New York Pathological Society October 8, 1931.

Clinical Diagnosis: Metastases to lungs and bones from a primary malignant tumor in the prostate or nasopharynx.

Laboratory Data: Blood pressure 112/70. Blood count: hemoglobin 72 per cent, white blood corpuscles 23,000, polymorphonuclear leukocytes 91 per cent. Sputum negative for tubercle bacilli. Urine negative. Blood Wassermann negative. Urea nitrogen 15 mg. per 100 cc., calcium 8.1 mg. per 100 cc., phosphorus 4 mg. per 100 cc. Roentgenograms of the skull and femur showed no evidence of metastases; the chest showed the lungs to be closely studded with metastatic newgrowths which varied in size from 0.5 to several cm.

The patient became progressively worse and died 5 days after admission.

AUTOPSY REPORT

Autopsy performed 12 hours postmortem. The body is that of a white male, 22 years of age, in complete rigor mortis, rather heavily built and well nourished. He has a moustache and a normal male distribution of facial, pectoral and pubic hair. The genitals are those of the normal adult male. The breasts are not enlarged. Bloody froth exudes from the mouth. No clubbing of the fingers is seen. The head and neck seem moderately swollen.

Abdomen: The panniculus adiposus is of normal thickness. The peritoneum is smooth and glistening and contains no excess of fluid. The situs viscerum is normal. The diaphragm reaches to the fifth rib on the right side and to the fifth interspace on the left.

Chest: No fluid or adhesions are found in the pleural spaces. Both lungs are crowded with large, firm, circumscribed nodules, varying in size from 0.5 to 3 cm. in diameter, with the majority about 2 cm. (Fig. 1). Those near the surface bulge outward, the intervening lung being depressed. Some show slight umbilications. Even throughout the pleura hemorrhagic spots are seen in some of the nodules. On section the nodules extending throughout the depth of both lungs are seen to be sharply circumscribed in the deeply congested and fleshy lung tissue. The cut nodules show an irregular mottling of grayish pink tissue with intervening vacuolated areas containing fluid blood. The larger branches of the pulmonary artery are normal. The trachea and bronchi contain bloody froth.

In the superior mediastinum (Fig. 1), just superior to the heart, an irregularly rounded mass of firm tissue 8 by 5 by 4 cm. is seen. The long axis is parallel to the trachea. Although adherent to the adjacent structures (pleural pericardium and vessels), it may be partly separated until it is revealed as lying between the superior

vena cava on the right, trachea and aorta posteriorly and the pericardium inferiorly. The two flaps of thymic fat adhere to the superior surface of the mass. There does not appear to be invasion of the adjoining structures by the tumor. Sections of the mass reveal an irregularly trabeculated surface similar to the cut surface of the pulmonary nodules. Small lakes of fluid hemorrhage are seen throughout the supporting trabeculations, which are grayish pink. Scattered throughout the mass are small circumscribed nodules of varying size. In the lower pole one of these nodules reaches the size of a pea and is distinctly circumscribed. It is grayish yellow in color and much firmer in consistence than the remainder of the tumor mass. Areas of necrosis and fibrosis are seen. At the inferior pole the hemorrhagic and necrotic tissue is replaced by firm white tissue with cyst-like spaces varying in diameter from 0.5 to 2 mm. This almost occludes the superior vena cava and grows upward into the left innominate vein.

Heart: The pericardium contains no excess of fluid. The superior portion of the parietal pericardium is reddish and slightly roughened where its external surface is adherent to the tumor mass. The subepicardial fat is normal in amount and distribution. The right ventricle is moderately dilated and hypertrophied. The columnae carnae and papillary muscles are prominent. The endocardium and valves are normal. The myocardium is firm and grayish red. The coronary arteries and their orifices are patent. The aorta is elastic and contains no plaques. The pulmonary arteries show a few intimal thickenings.

Liver: Weight 2020 gm. It is smooth and of normal consistence. The central lobular areas show a deep brownish red color. No metastases are found.

Pancreas: Normal.

Spleen: Weight 275 gm. The cut surface shows a deep red pulp in which the follicles are large and prominent.

Genito-Urinary Tract: Both kidneys together weigh 325 gm. They present a normal appearance. The pelves, ureters, bladder and prostate are normal. The testes are slightly smaller than normal. The epididymis and testes contain no nodular enlargements or areas of hemorrhage. The rete and vas deferens are grossly negative, presenting no tumors or areas of hemorrhage. The seminal vesicles show no abnormality.

Adrenals: Normal.

Gastro-Intestinal Tract: Normal.

Bone Marrow: Normal.

BIOLOGICAL EXAMINATION

Twelve hours postmortem:

Urine: A positive Aschheim-Zondek test was obtained in four mice with quantities of urine varying from 1.5 to 2.4 cc.

Tumor Tissue (Lung Metastases): Ether extract for the female sex hormone (Frank) was negative with 20 gm. of tissue.

Alcohol extract for the anterior pituitary hormone with 1 cc. equivalent to 9.5 gm. of tumor tissue: two mice gave positive results with 1.2 cc., one was negative with 0.5 cc.

MICROSCOPIC EXAMINATION

Tumor Mass: In its inferior portion many types of epithelial structures are seen. They vary from solid nests of undifferentiated epithelial cells to cyst-like spaces lined by squamous epithelium. There are gland-like structures with low cuboidal, high columnar, and pseudostratified epithelium. Occasionally the high columnar epithelium is ciliated (Fig. 2).

The remainder of the tumor mass is almost entirely necrotic. At the periphery of the necrotic areas cell masses with deeply staining acidophilic cytoplasm appear in syncytial arrangement. The nuclei are irregular in form and are hyperchromatic. The cytoplasm appears in masses, elongated strands and ramifying networks. Isolated cells and multinucleated giant cells are also seen, especially in the necrotic areas. Plaques of cells with definite boundaries and vacuolated dust-like cytoplasm are present. Their nuclei are vesicular, and have a definite nuclear membrane with one or more coarse chromatin clumps (Fig. 3). The proportions of the two types vary; frequently the plaques of cells are completely surrounded by the syncytially arranged acidophilic cytoplasm. The plaques of cells are identical with the Langhans cells seen in the uterine and in the testicular chorionepithelioma. The syncytial cytoplasm appears to be more hardy since it occurs alone in the midst of necrotic areas. That these are not advancing cytoplasmic columns may be surmised from

the presence of degenerating and degenerated cell plaques. Occasionally cilia-like projections are seen on the syncytial cytoplasm. Both types of cells are seen in the small veins, occasionally even where the wall has been destroyed and a small thrombus occludes the vessels. Calcific foci are seen in the necrotic areas. Glycogen stains give negative results (tissue fixed 12 hours after death).

Thymus: Shows normal structures with increased amount of fat in the trabeculae. Occasional veins in the fat and connective tissue show syncytial cytoplasm similar to that seen in the masses.

Lungs: The nodules in the lungs show complete central necrosis surrounded by alternating areas of necrosis and hemorrhage. Around this zone is another with hemorrhage, necrosis and bands of acidophilic cytoplasm with hyperchromatic nuclei in single cell and syncytial arrangement. External to this there are plaques of cells with definite cell boundaries, vacuolated or dust-like cytoplasm and round or oval vesicular nuclei with sharp nuclear membranes and one or more coarse chromatin clumps. Mitoses are seen. Covering these plaques, identical with the Langhans cells of the primary mass, are the syncytial masses of acidophilic cytoplasm with hyperchromatic, irregularly shaped nuclei. Mitotic figures are frequent. Occasionally, under oil immersion magnification, cilia-like projections are seen on the syncytial cytoplasm. The picture is identical with the typical chorionepithelioma of Marchand. The boundaries of zones are not rigid but merge indefinitely with each other. In none of many lung sections are teratomatous elements present.

Zones of compressed lung tissue surround the nodules. The pulmonary arteries contain syncytial masses of cytoplasm. Alveoli filled with fibrin, polymorphonuclear leukocytes and pigment-laden phagocytes are seen, with occasional polymorphonuclear leukocytes and fibrin-filled bronchi.

Lymph Nodes: Neither the thoracic nor the retroperitoneal nodes show tumor metastases. There is sinus endothelial hyperplasia with marked erythrophagocytosis.

Testes: Both testes were cut in 2 mm. blocks and embedded. The entire testes were thus cut. Slides were cut from each block. No tumor nodules or scars were found. A marked hyperplasia and hypertrophy of the Leydig cells is present throughout both testes (Fig. 4). There are nodules of interstitial cells which reach 500 microns in diameter. The tubules show spermatogenesis. Numerous

atrophic tubules are present. Within their lumens are bodies with concentric rings of calcification. The rete and epididymis show no changes.

Prostate and Seminal Vesicles: Sections through the prostate show adenomatous hyperplasia with papillary proliferations in the lumen. There are no tumor nodules.

Liver: The central lobular areas show congestion with atrophy of the liver cell cords.

Spleen: The follicles are normal. There is slightly increased cellularity of the pulp with increase in the polymorphonuclear cells. The sinuses and pulp spaces contain much blood. The sinus walls are prominent, the intersinusoidal spaces are widened and the cytoplasmic reticulum is conspicuous.

Kidneys: Marked congestion of the cortex and medulla is present.

Pancreas, Adrenals, Thyroid: No changes are seen.

Breast: Tissue taken from immediately beneath the right nipple shows only fat and connective tissues with nerves and blood vessels. No glandular structures are seen.

DISCUSSION

Cases of extragenital chorionepithelioma in males have been published by Bostroem in 1902, Ritchie in 1903, Askanazy in 1906, Bonney in 1907, Fischer in 1908, Nakayama in 1910, Weber in 1918, Lambert and Knox in 1920, Miller and Browne in 1922, Krassnianskaya in 1929, Schultze in 1930, Arendt in 1931, and Heaney in 1933. A critical examination of their protocols suggests the possibility that a primary origin from the testicle may have been overlooked in a number of instances.

Bostroem himself now doubts the interpretation originally published for his case of intracranial chorionepithelioma, since he believes an incomplete examination of the testicles was made.

Askanazy's case of pineal teratoma had no testicular examination.

Frank's Case III, with a mediastinal mass, had no testicular examination. There was also an incomplete examination of the mediastinal tumor, since only necrotic tissue was found.

The omental tumor and its metastases in the case described by Bonney showed only chorionepitheliomatous tissue. The testicles were incised and found negative grossly. This observation was not controlled by histological examination.

Fischer found a retroperitoneal tumor in a male with an absent right testis. He was probably dealing with an abdominal testicle with chorionepitheliomatous neöplasia.

Nakayama observed a patient with a retroperitoneal chorionepithelioma demonstrated at the operating table. There was no examination of the testes.

Weber's patient had a retroperitoneal tumor with liver and lung metastases. Portions of organs were sent to the author for examination. The lumbar lymph glands made up the mass. No teratomatous elements were noted. One should hesitate to accept the conclusions drawn by the author since he was the recipient of incomplete postmortem material.

Schultze considered a nodule in the right testicle to be a metastasis from a primary retroperitoneal tumor, an obviously incorrect interpretation.

The statistics of Greiling compiled from a study of 220 cases of malignant tumors of the testicle with metastases show retroperitoneal involvement in 100 per cent of the cases. Such figures justify one in doubting the validity of the reported cases of primary retroperitoneal chorionepithelioma with no or incomplete testicular examinations. The fact that brain metastases occur in 5.9 per cent of the cases also throws doubts on the reports of cerebral chorionepitheliomas without testicular examinations. These considerations lead me to a critical review of the remaining 6 cases.

In the cases of Ritchie and of Lambert and Knox there were present tumors containing teratoid and chorionepitheliomatous elements. In both cases the tumors were situated in the anterior mediastinum (a location not mentioned by Greiling in any of the 220 cases with metastases). This similarity to the tumor here reported would suggest the possibility that there were cases of primary extragenital chorionepithelioma in males, were it not for the absence of testicular examinations. In this connection the experiences of Prym and Ewing make one hesitant in accepting cases of this type. The former, in 1927, described a case of chorionepitheliomatous metastases from a small tumor of the right testis which had healed spontaneously. Microscopic examination of the testicular nodule revealed only elastic and connective tissue fibers. The common experience with spontaneous regression of female chorionepitheliomas should lead one to accept Prym's interpretation. Ewing has seen "a medi-

astinal teratoma secondary to a small testicular growth which escaped several examinations during life." He writes further: "Some reported abdominal and thoracic teratomas may possibly represent metastatic growths from minute teratomas of the ovary or testis."

Miller and Browne in 1922 reported a retroperitoneal chorionepithelioma in a male. Microscopically the tumor was a typical chorionepithelioma. Both testicles were found to be normal after careful examination. There were metastases in the retroperitoneal lymph nodes and liver.

Krassnianskaya in 1929 reported a primary tumor at the hilum of the left lung. There were generalized metastases in the spleen, kidneys, retroperitoneal lymph nodes, adrenal glands and brain. The prostate was small but normal. Both testicles were in the scrotum. Very thin sections of both testicles and epididymi showed no changes, grossly or microscopically. The tumor and its metastases showed the characteristic picture of the chorionepithelioma. The primary tumor and its metastases showed no evidence of a teratoma. On this basis Krassnianskaya believes that he has observed a pure chorionepithelioma in an extragenital location in a male. He attempts to divide the extragenital chorionepithelioma into two types: those that are pure and those that contain teratomatous elements. This classification I believe to be fallacious in view of the difficulty in determining the previous presence or absence of teratomatous elements. The experiences with chorionepithelioma of the testicle demonstrate how readily the components of a teratoma may be destroyed by the invading chorionepitheliomatous elements.

Arendt, in 1931, described a mediastinal teratoma with chorionepitheliomatous metastases in the lungs and liver. His patient was 20 years of age and had complained of bloody sputum 2 months before admission. Gynecomastia was present. On physical examination the testicles were hard and about 2 by $1\frac{1}{4}$ cm. in diameter. At autopsy a tumor about 3 cm. in diameter was found in the anterior mediastinum. It extended into the left lung and pericardial sac. Grossly the tumor resembled a chorionepithelioma. Both testicles were grossly atrophic. Microscopically the testes showed an atrophy of the tubular elements with a marked proliferation of Leydig cells. There were no biological tests performed. The presence of a mediastinal tumor with chorionepitheliomatous metastases in the lungs

and liver with negative testes (grossly and microscopically) appears to answer the strictest criteria for the presence of primary extragenital chorionepithelioma in a male.

Heaney in 1933 observed a male, aged 40 years, with a large retroperitoneal tumor. The patient died subsequent to an operation for the removal of the mass. The anatomical diagnosis was that of primary retroperitoneal chorionepithelioma probably derived from the urogenital anlage. The inferior vena cava and left renal vein were filled with tumor tissue. There were metastases in the lungs. The right testicle was atrophic. Microscopically the tumor closely resembled a typical chorionepithelioma. There were no teratomatous elements present. The left testicle showed a hyperplasia of the interstitial cells. An area of new hemorrhage, blood pigment and fibrin was found. This is interpreted by the author as probably the result of an old trauma. No evidence of tumor tissue was found. There was no retroperitoneal lymph node involvement. The author was fully aware of Prym's stand and states that "testicular involvement has been excluded with certainty in this case."

In summary it appears that the cases of Miller and Browne, Krassnianskaya, Arendt and Heaney appear to fulfill the criteria necessary for the diagnosis of a primary extragenital chorionepithelioma in a male.

The case here reported belongs in a similar category. The presence of a teratomatous tumor in the mediastinum with metastases to the lungs and the absence of tumor, active or healed, after careful macroscopic or microscopic examination of the genital tract, including the prostate, seminal vesicles, vas deferens and testes, should be sufficient to justify this conclusion, especially when one considers that both testes have been cut and blocked in their entirety in 2 mm. sections. Furthermore, the absence of metastatic chorionepithelioma below the diaphragm, grossly and microscopically, also the absence of thoracic and retroperitoneal lymph node involvement speak against the presence of a focus other than the anterior mediastinum. Additional support to this view is furnished by the observations of Smith, who has collected 147 cases of mediastinal dermoid cysts and teratomata with 12 showing malignant neoplastic growth. Thus one can see no reason, on theoretical grounds, why a mediastinal teratoma cannot have chorionepitheliomatous elements. The microscopic examination of the primary tumor and the pul-

monary metastases shows a structure identical with the typical chorionepithelioma of Marchand. The acidophilic syncytium, the plaques of Langhans cells, the hemorrhagic character, and the areas of necrosis all indicate that it is identical with the Marchand chorionepithelioma. The microscopic picture coincides, too, with the descriptions of testicular chorionepithelioma by Wlassow, Risel and Schlagenhauser.

Biologically the case presented is similar to the uterine and testicular chorionepitheliomas. The tissue extracts and urine gave positive Aschheim-Zondek tests. The female sex hormone test of Frank was negative. Increasing numbers of positive Aschheim-Zondek tests in testicular chorionepithelioma have been reported. Heidrich, Zondek, Kriss, Hady, Ehrhardt and Frank report positive results. Ferguson reports positive Reaction I (prolan A) tests with testicular teratoma and the embryonal carcinomas of the testis (seminoma of Chevassu). Thus far one may agree with Heidrich, Fels and Mathias that certain neoplasms of the testis cause the excretion of a hormone whose presence in the urine and, one may add, in tumor tissue extracts, can be detected readily by biological means. The positive results reported with certain genital and extragenital malignant growths are not the Reactions II and III of Zondek, but merely Reaction I. The latter result may be obtained with rapidly proliferating tumors, in diminished genital function at the beginning of the menopause and in certain types of amenorrhea. It consists of cystic enlargement of the ovaries in immature rodents usually combined with oestrus production and follicle growth. It is not indicative of pregnancy. Reactions II and III show hemorrhagic follicles (blood points) and corpus luteum formation, respectively. Teratoma testis with chorionepithelioma and the embryonal carcinoma of the testis give Reactions II and III. Reaction I (prolan A) is found in conjunction with too many conditions to be of aid in the diagnosis of testicular tumors, although its importance in the determination of the extent of the disease and the effect of treatment for teratoma testis is amply demonstrated by Ferguson.

An interesting observation is that of the marked hypertrophy and hyperplasia of the interstitial cells of the testes. This was marked throughout the testes, resulting in diffuse strands and nodules up to 500 microns in diameter. Arendt and Heaney also report a marked proliferation of Leydig cells with almost tumor-like growths. Wlas-

sow, Risel, Dillman and Hedinger have also observed the marked proliferation of the Leydig cells in testicular chorionepithelioma. The occurrence has been noted in other conditions but its significance remains unknown.

Gynecomastia has been reported with teratogenous chorionepithelioma. It was not present in this case.

SUMMARY AND CONCLUSIONS

A case report with autopsy findings in a male aged 22 years with a primary teratoma of the anterior mediastinum containing chorionepitheliomatous elements is presented. The tumor invaded the superior vena cava, studding both lungs with chorionepitheliomatous nodules.

Careful gross examination revealed no metastases in the other organs or lymph nodes. The genital tract (testes, vas deferens, seminal vesicles and prostate) showed no tumor nodules. The testicles were sectioned in 2 mm. blocks and slides were made from each block. Examination revealed no tumor nodules.

Microscopic examination of the tumor revealed teratomatous and chorionepitheliomatous elements. Only chorionepithelioma was found in the pulmonary metastases. The testes showed no neoplastic elements. Marked interstitial cell hyperplasia of the testes was seen.

These observations refute the contention of Prym and Oberndorfer, the latter writing "dass beim Mann das Chorionepitheliom immer mit Keimdrusengeschwülsten in Zusammenhang stehen muss."

The Aschheim-Zondek test was positive in both the urine and tumor tissue extracts.

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DESCRIPTION OF PLATES

PLATE 130

FIG. 1. Tumor mass in the superior mediastinum with erosion into the left innominate vein. Lungs with metastatic nodules.

FIG. 2. Teratomatous structures in the inferior pole of the mass.



I

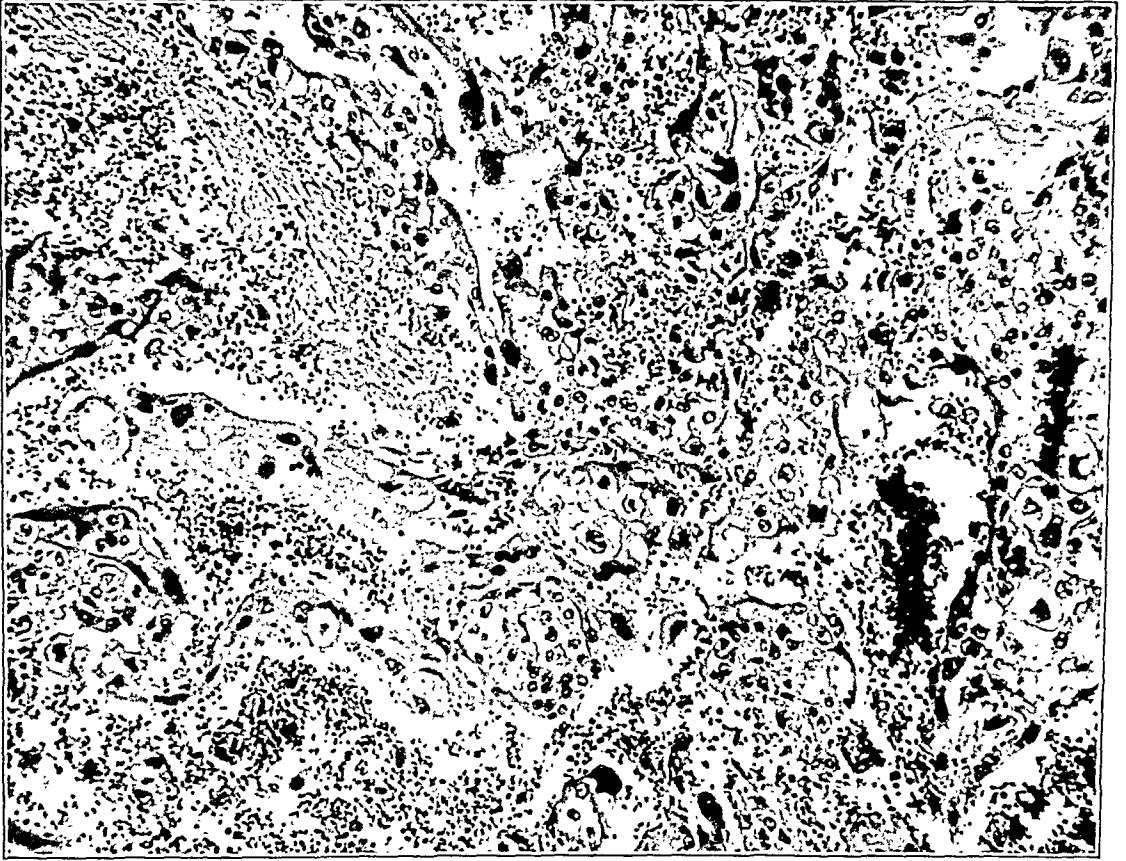


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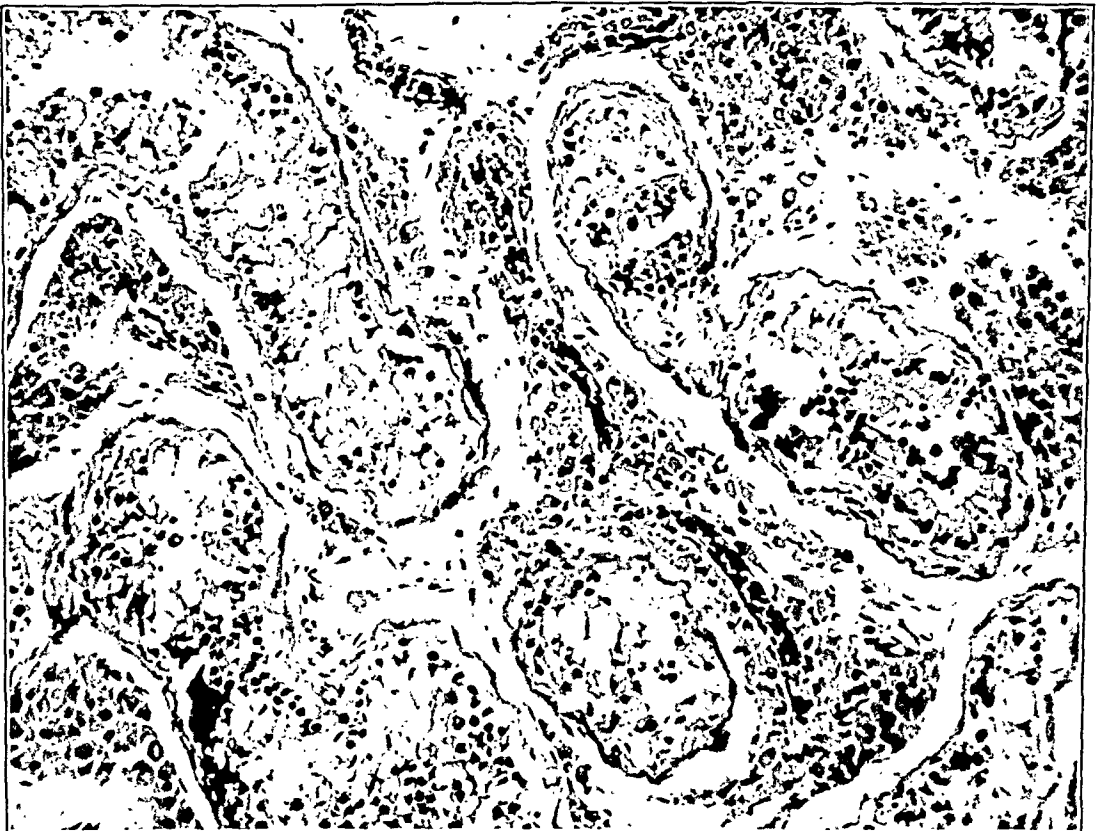
PLATE 131

FIG. 3. Plaques of Langhans cells with syncytial cytoplasm, from the upper pole of the mass, representing elements of typical chorionepithelioma.

FIG. 4. Hyperplasia and hypertrophy of the interstitial cells of the testis.



3



4

MULTIPLE HEMANGIOBLASTOMAS OF THE SPINAL CORD WITH SYRINGOMYELIA *

A CASE OF LINDAU'S DISEASE

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In 1926 Lindau described a syndrome whose chief feature was an angiomas of the central nervous system. Brandt and Berblinger had previously noted this disease entity but had failed to describe it thoroughly or to emphasize its distinctness. In this condition cystic or solid hemangioblastomas of the cerebellum, brain stem or spinal cord, single or multiple, were found associated with angiomas of the retinae (von Hippel's disease) and concomitant malformations or tumors in the somatic organs. The latter were described as cystic pancreas, cystic kidneys, cysts of the liver, hypernephromas and adenomas of the kidneys and adrenals, and cavernomas of the liver. Lindau described 15 cases in all. Ten were collected from the literature and had been variously described and classified. Five were cases of his own. He showed that there was a distinct familial incidence of the disease and that the average age of onset of symptoms was about 30 years. In 4 of the 15 cases hemangioblastomas of the cord were present (Wersilow, Brandt, Tannenberg and Koch). In all 4 the cord tumors were multiple and in 3 were associated with lesions outside the nervous system, of the type described above (Brandt, Tannenberg and Koch). In only 1 case was there an associated syringomyelia of the cord (Tannenberg). Since Lindau's report 4 more cases of hemangioblastoma of the spinal cord have been described (Guillain *et al.*, Schuback, Kernohan *et al.*, and Russell). Two presented lesions characteristic of Lindau's syndrome (Schuback, and Kernohan *et al.*). There are thus only 3 cases of Lindau's disease in the literature to date in which hemangioblastomas of the cord were associated with syringomyelia. The following is the report of a 4th case.

* Received for publication April 5, 1934.

REPORT OF CASE

Clinical History: E. W., (P. H. No. 312177), male, 33 years of age, was admitted to the Presbyterian Hospital complaining of numbness in the arms and legs and griping pains in the abdomen.

His father had died of brain tumor at the age of 36 and his mother of "spinal meningitis" at 28 years of age. A twin brother and sister had died at birth.

Twelve years prior to his present illness the patient struck his head while diving and was unconscious for a short time. Detachment of the right retina occurred and he underwent twelve operations until the right eye was finally enucleated 6 years prior to the present illness. Two years later detachment of the left retina occurred and that eye was enucleated. This was unassociated with further trauma.

During the year before admission the patient developed slight unsteadiness on his feet with a tendency to fall to the right, and tremor of the hands on performing skilled acts. Constipation, which had been present for years, grew increasingly severe. There were recurrent attacks of vertigo. Numbness of the right arm and leg and of the left foot developed 5 months before admission. Severe griping and stabbing pains occurred, starting at the umbilicus and radiating to the flanks, accompanied by a compressive girdle sensation. He grew progressively weaker, losing 13 pounds in the last few weeks before his entry to the hospital.

On admission he was quite weak, and gait and station could not be tested. All the coordination tests were inconclusive for the same reason. There was great weakness of all the muscles of the four extremities, more marked on the right side. Hyperreflexia was found in the left upper and both lower extremities with increased muscular tone. The upper abdominal reflexes were diminished and the lower abolished. The Babinski test was positive on the right and questionable on the left. The interossei muscles of the right hand, all the muscles of the right forearm, the right biceps, triceps, supra- and infraspinati and posterior portion of the deltoid muscles showed a well marked atrophy. On the left there was moderate atrophy of the interossei and slight atrophy of the forearm muscles and supra- and infraspinati. Direct muscular irritability was definitely increased in the atrophic muscles.

Vibratory sensibility was slightly impaired below the patellae in both lower extremities. The sense of position was slightly diminished in the toes. Perception of pain and temperature was completely lost from the eighth thoracic segment up to the fourth cervical on each side. It was considerably diminished up to the first cervical segment on the right and third cervical on the left, and moderately diminished down to about the first lumbar segment. Touch was only slightly impaired, if at all. There was difficulty in swallowing, which had developed recently, and questionable fibrillations of the tongue.

The spinal fluid pressure was 150 mm. Compression of the jugulars caused a rise to 190 mm. with a slow fall. Coughing brought the pressure to 240 mm. and straining to 300 with a very gradual drop. The fluid was xanthochromic, showed a 2 plus globulin, contained 5 cells and gave a 4 plus Wassermann test. The colloidal gold test resulted as follows: 111112210. The blood Wassermann was negative.

The picture that the patient presented was thus typical of intramedullary disease of the spinal cord. The course seemed rapid for syringomyelia. The

diagnosis of medullary tumor was considered, but the positive spinal fluid Wassermann made it seem more probable that a gumma was present or that there was luetic thrombosis of the spinal arteries.

The patient developed an intractable hiccoughing a few days before death, and finally succumbed to a terminal bronchopneumonia.

GROSS AUTOPSY FINDINGS

Brain: The dura was somewhat tense, smooth and glistening throughout, and the dural sinuses were patent. The cerebral hemispheres were symmetrical. There was mild generalized flattening of the gyri and narrowing of the sulci. The pia was thin and translucent throughout. On section a mild dilatation of the lateral ventricles was noted. No lesions of the parenchyma were found.

The cerebellum appeared negative externally. On section a small, oval, smooth-walled cyst containing gelatinous yellowish fluid and measuring 17 by 10 by 5 mm. was found in the posterior portion of the central white matter of the lower half of the left cerebellar hemisphere. In the floor of the cyst a small mural nodule was found. It was soft, yellowish brown, had a rather smooth surface and measured 5 by 3 by 2 mm.

The greater portion of the brain stem appeared normal externally. The lower portion of the medulla from about the middle of the inferior olives downward was considerably increased in size, its diameters being almost twice the normal. The increase was greatest where the stem joined the cervical cord. The pia over the base of the stem was slightly clouded. A mild dilatation of the aqueduct of Sylvius and anterior portion of the fourth ventricle was noted on section. In the lower half of the dorsal portion of the medulla a ragged oval cavity was found containing clear yellowish fluid and measuring 4 by 11 mm. in cross-section at the junction of medulla and cord. It ended in the first cervical segment and its total length was 14 mm. About it was a narrow band of grayish translucent tissue varying in width from 0.5 to 1 mm. Most of the structures in this portion of the medulla were not identifiable grossly and the tissue had a grayish white, scarred appearance.

Spinal Cord: The pia was clear but the pial vessels were quite hyperemic. The cervical cord was considerably enlarged and this was most marked in the first four segments. Here it measured approximately 24 mm. transversely and 13 mm. anteroposteriorly. On

the dorsal surface of the second and third cervical segments to the left of the midline a flat, yellowish, soft tumor mass measuring 6 by 5 by 3 mm. was found (Fig. 1). Its margins were fairly sharp and it extended down into the posterior columns on the left side to within a short distance of the central canal. The white and gray matter at its margins was edematous, somewhat yellowish and softened. The outlines of the gray matter were indistinct at this level. Both gray and white matter had either a yellow or grayish white tinge and seemed edematous. Small zones of softening without cavitation were found in the posterior horns and occasionally in the anterior at the level of the tumor and above it up to the medullary cavity, which was described above as ending in the first cervical segment. In the fourth and fifth cervical segments single and multiloculated cavities were found involving the dorsal columns and posterior horns and lying dorsal to the central canal (Fig. 4). These were of the same character as the larger cavity in the medulla. They grew less extensive and numerous in the succeeding lower segments and ended in the second thoracic segment. The demarcation between white and gray matter became increasingly distinct and the cord grew less edematous. The rest of the thoracic cord looked somewhat irregular in caliber, but was of normal consistence. There was slight brownish discoloration of the posterior columns in the lower thoracic segments with petechial hemorrhages and small softened zones in the gray matter, particularly the posterior horns. In the tenth thoracic segment a small, grayish, granular tumor nodule 2 mm. in diameter was found in the right posterior horn (Fig. 2). At this level again there was edema and lack of clarity of the margins of the gray matter. In the next segment below this multiple central cavitations reappeared and gave place to a single large cavity in the first lumbar segment. This latter involved portions of the gray horns on the right, as well as parts of the white columns. It ended in the fourth lumbar segment and was 6 by 4 mm. at its widest point (Fig. 3). The walls of these lumbar cavities were wider, firmer, paler and more fibrous than that of the medullary cavity and varied in width from 1.5 to 2 mm. Their contents were the same as that of the bulbar cavity. Throughout this zone and for a short distance below it the gray and white matter had no very clear outlines. On the dorsal surface of the cauda equina and attached loosely to the fourth and fifth lumbar roots on the left was a small, yellowish, en-

capsulated tumor nodule measuring 4 mm. in diameter and resembling grossly the tumor in the cervical region.

Left Adrenal: The left adrenal was enlarged and almost spherical in shape, weighing 60 gm. On section a large, roughly spherical, moderately firm tumor, measuring 4.5 by 4 by 4 cm. was found within the gland. It was variegated in color, ranging from pinkish gray to yellowish brown, and was studded with many thin-walled blood vessels. It appeared to have arisen in the medulla and to have displaced the cortical tissue at its margins, so that only a thin rim of cortex intervened between the periphery of the tumor and the slightly thickened adrenal capsule. At either pole the normal adrenal tissue was better preserved. At the lower pole two smaller oval-shaped masses measuring 1 and 1.5 cm. in diameter, respectively, similar in all respects to the larger mass, were found. The right adrenal showed no abnormalities. A paraortic lymph node in the region of the left adrenal contained two small, irregular yellowish zones in its substance.

Kidneys: The kidneys were normal in size and appearance save for the presence of several tumor nodules. The largest of these measured 2 by 1.5 by 1 cm. and was situated on the posterior aspect of the right kidney near the hilum. Externally it appeared as a firm, grayish white mass embedded in the cortical surface. The cortical tissue at the margins of the mass was somewhat depressed and scarred. On section the mass was seen to be composed of an outer white fibrous shell measuring 2 mm. in diameter, and a central core made up of reddish and yellowish softer tissue in which a few small cystic spaces 1 to 2 mm. in diameter were present. The nodule extended into the medulla to a slight extent.

Beneath a small pit-like depression on the lateral aspect of the left kidney a small, firm, grayish white nodule measuring 3 mm. in diameter was seen. This contained two small, reddish foci. It resembled the larger nodule in the right kidney somewhat. A third nodule 1 cm. in diameter bulged above the cortical surface near the upper pole. It was composed of soft, grayish brown tissue, was encapsulated and contained several tiny cysts.

Pancreas: The pancreas was normal in size and shape. Several small, thin-walled cysts filled with clear fluid and situated close together were found in the body of the organ. The largest measured 3 mm. in diameter. The pancreatic duct was of normal caliber.

There was an anatomically patent foramen ovale. The liver contained no cysts nor did it show any other abnormalities.

In addition to the above findings there were bilateral lobular pneumonia, acute fibrinous pleurisy, and an acute splenic tumor.

MICROSCOPIC EXAMINATION

Tumor in Upper Cervical Cord: On the dorsal side of the cord, to the left of the midline, a highly vascular, moderately cellular tumor mass is found displacing a good portion of the posterior columns and distorting the cord considerably. It is fairly well demarcated. The predominant elements in its structure are blood vessels, capillary in type and fairly irregular in size and shape. Occasionally these lie approximated, but most frequently they are separated by one or more layers of intervening cells (Fig. 5). Some larger vascular channels are also present but their walls are composed of collagenous connective tissue with no evidence of arterial or venous structure. From these the surrounding capillaries are often found to radiate (Fig. 9). The capillaries, as noted above, are quite irregular with much branching and outpocketing. They are lined by a single layer of endothelium. This varies from flat, elongated cells with oval, dark, concentrated nuclei, to full-bodied epithelioid-like cells with oval, vesicular nuclei. These frequently seem to merge intimately with the intervascular elements. There is no endothelial hyperplasia, however, nor are any mitotic figures noted in the cells of the capillary walls. Their lumens are either empty or contain varying numbers of erythrocytes. In none of the vessels are any immature blood cells noted. The walls of the larger channels described above are at times quite thick and occasionally hyalinized. The cellular elements found between the vessels are chiefly large-bodied, polygonal and spherical with vacuolated, foamy cytoplasm (Fig. 7). The nuclei are comparatively small, concentrated, often compressed at the periphery of the cell and so lunate-shaped. In other cells again the nuclei are more centrally placed and angulated, presenting concave surfaces due to compression by the lipid globules. Where these lipid-laden cells are particularly abundant and most heavily vacuolated, the capillaries between them are compressed, though numerous, and at first glance seem to form only an insignificant portion of the picture (Fig. 8). The lipid material in these cells is found to be a mixture of neutral fats and finer lipoids which stain

positively with Nile blue sulphate. Among these large-bodied cells are less obvious, elongated cells with oval, deeply staining nuclei. These cells appear to be endothelial in type and as noted above are occasionally seen to be intimately associated with the cells lining the capillaries. A few scattered, rounded cells, somewhat smaller than the vacuolated cells, are found to be multinucleated. Occasional small cysts filled with pink granular material are seen, as well as evidence of more generalized edema such as the separation of individual cells by similar material.

There is an abundant reticular network throughout the tumor outlining the capillaries, bridging between them, and running to the collagenous walls of some of the larger channels (Fig. 9). The latter contain no elastic tissue or any smooth muscle cells. No nervous or glial elements are found in the growth.

The tumor is apparently attached to, and growing into, the pia externally (Fig. 1). Internally, although it has a definite margin, it has no capsule and numerous capillaries of the neoplasm extend beyond its general border into the surrounding nervous tissue. Directly about the tumor the parenchyma is quite edematous and markedly degenerated and here there is considerable proliferation of microglia with the formation of compound granule cells. Beyond this zone of degeneration is an area in which there is hypertrophy and proliferation of astrocytes with multiplication of glia fibers. This zone is predominantly in the posterior columns and horns. The white matter of the lateral and anterior columns, especially the former, shows a considerable diffuse loss of myelin sheaths and axones with the presence of scattered compound granule cells. On the left side the anterior horn cells have almost all disappeared while on the right a good number are preserved.

Mural Nodule of Cerebellar Cyst: This growth also is quite vascular. Its vessels in general appear more regular and many are considerably more dilated, although all appear to be capillary in type. In some zones these vessels lie closely approximated and are packed with erythrocytes. In the more cellular areas the intervacular elements are predominantly polygonal, large-bodied or elongated narrow cells with concentrated oval nuclei. None of the lipoid-containing cells are seen here. There is considerable interstitial edema, as evidenced by the separation of the cells. The neoplasm, although fairly well circumscribed like the first lesion, shows the same minor

surface irregularity. The surrounding cerebellar tissue shows a moderate gliosis and some edema.

The wall of the cyst is lined by a narrow band of glia fibers. The cerebellar parenchyma external to it shows some signs of compression.

Tumor Attached to Cauda Equina: This is similar in all respects to the neoplasm in the cervical cord. It is surrounded by a narrow, collagenous connective tissue capsule.

Tumor in Thoracic Cord: This growth is somewhat more cellular than that in the cervical cord and correspondingly less vascular. Its vessels are all capillaries. The interstitial cells are only occasionally foamy. The majority are elongated or fusiform and have oval, deeply staining nuclei. The tumor is fairly small, well circumscribed and lies in the right posterior horn. Its margins are similar to those of the cervical lesion. About it there is a fairly wide zone of edema, mild degeneration and gliosis which includes most of the gray matter and the adjacent areas in the white columns.

Syringobulbia: Lower Medulla Near Junction with Cord: The pia is somewhat thickened and contains occasional lymphocytes. The stem is much increased in size and its architecture markedly disturbed. A large, roughly oval cavity with a number of narrow out-pocketings is found occupying the central portion of the dorsal half of the stem. It lies just ventral to the obliterated central canal which is represented by a double row of polygonal and irregularly cuboidal cells. Its wall is a dense mass of glia fibers, heavier internally and less concentrated externally. Only occasional glia nuclei can be found in the central layer, whereas externally a fair number of fibrous astrocytes are present. The glia fibers, for the most part, run parallel with the margin of the cavity. The external layer contains a moderate number of hyperemic capillaries and one arteriole shows a perivascular infiltration of lymphocytes. There is considerable edema of the surrounding parenchyma with degeneration of ganglion cells, myelin sheaths and axones and scattered compound granule cells are present. A diffuse gliosis is observed in this zone. The external glial membrane is moderately thickened.

Microscopically this syrinx is found to reach down to a point on a level with the beginning of the tumor of the cervical cord.

Syringomyelia: The cavities found beginning just below the intramedullary cervical growth are microscopically similar in most re-

spects to those in the medulla. Their walls show a similar conformation and the parenchyma about them shows varying degrees of the same edema, degeneration and gliosis. This is true also of the extension of these cavities into the upper thoracic cord. In each case they do not seem to involve the central canal which is everywhere obliterated and represented only by irregular masses of ependymal cells, as in the lower medulla.

The syringomyelic cavities appearing just below the thoracic tumor vary somewhat from the above. Their walls are considerably thicker. The inner layer is composed of a much looser, edematous mass of glia fibers in which no cells or even nuclei can be discerned. External to this is a wide band of densely woven glia fibers containing scattered glia nuclei (Fig. 3). Outside this layer is an irregular zone of gliosis in which a fair number of fibrous astrocytes can be discerned. The parenchyma about these cavities shows more gliosis and less edema than is seen about the cervical and upper thoracic cavities.

Tumors in Left Adrenal: The three neoplastic masses are identical in appearance. They are composed of large, irregularly polygonal cells with indistinct cell outlines and large, round or oval, pale staining vesicular nuclei. These nuclei are rather uniform in appearance and have several poorly defined nucleoli. No mitotic figures are seen. The cells are arranged in small cords and nests which sometimes simulate alveoli. They are embedded in a rich vascular plexus, many small, freely anastomosing capillaries coursing about small groups of cells. In Zenker-fixed material the cytoplasm of the tumor cells is made up of finely granular, brown-staining matter, strongly resembling chromaffin. In formalin-fixed material this brown pigmentation is not seen. There are no areas of degeneration or necrosis in the tumor tissue although a few small hemorrhages and foci of small round cells are present. The tumor nodules are not encapsulated but are sharply limited from the surrounding adrenal cortex. No fat is demonstrated by sudan III stains. The Foot-Bielschowsky staining method reveals the presence of many fine reticulin fibers about the nests of cells.

Kidneys: The large white mass in the *right kidney* is composed of dense, avascular, pink-staining scar tissue containing a few scattered connective tissue nuclei, some large solid masses of calcium and several central cystic areas. The latter are not lined by epithelium,

but their walls are irregular and fibrous. They contain only a few extravasated erythrocytes.

One of the small masses in the *left kidney* resembles the large mass in the right, being composed of dense fibrous tissue. It contains several small cysts lined by flattened epithelium.

The second nodule in the left kidney is entirely different from the preceding two lesions. It is vascular and is composed of irregular cords of polygonal cells and tubular structures, many of which are filled with red blood cells (Fig. 10). A good number of the tubular structures are greatly distended and cystic. They are lined by a single layer of uniform cuboidal cells. A thin fibrous capsule surrounds most of the mass but in one area it is directly continuous with the adjacent renal cortex. In one part of the nodule many elongated, cylindrical cells with vacuolated cytoplasm are present.

Paraortic Lymph Node: In this lymph node, taken from the region of the left adrenal, are found two small masses of polygonal cells arranged in irregular cords (Fig. 11). These have foamy, lipoid-containing cytoplasm and round or oval, deeply staining nuclei. The cells strongly resemble normal adrenal cortical cells. No pigment is present in the cytoplasm of these cells.

Pancreas: The cysts in the pancreas are lined by flattened epithelium and encircled by a narrow band of fibrous tissue (Fig. 12). They contain small amounts of pink-staining granular debris. The acini immediately adjacent to the cysts are compressed and atrophic.

Summary of Autopsy Findings: Autopsy revealed a small cystic hemangioblastoma situated in the posterior portion of the central white matter of the right cerebellar lobe; a mild internal hydrocephalus; three hemangioblastomas of the spinal cord; syringobulbia and syringomyelia; congenital cysts of the pancreas and kidneys; a benign hypernephroma of the left kidney; an adrenal rest in a retroperitoneal lymph node; three paragangliomas of the left adrenal and a patent foramen ovale.

There was a terminal lobular pneumonia with acute fibrinous pleurisy and an acute splenic tumor.

Comment: Unfortunately we were unable to obtain any detailed clinical facts, pathological reports or specimens concerning the enucleated eyes beyond the information that they had been enucleated for detached retinæ with terminal glaucoma. Almost all cases of angiomas of the retina end in retinal detachment which finally

necessitates enucleation of the eye. A majority of the cases of angiomas of the central nervous system, in the sense of Lindau's disease, are accompanied by angiomas of the retinae and it is extremely likely that the latter condition was present in our patient.

DISCUSSION

Hemangioblastomas of the central nervous system are uncommon. They are most apt to occur in the third and fourth decades of life. The cerebellar hemangioblastomas comprise somewhat less than 1 per cent of intracranial tumors. Similar lesions of the spinal cord, stem and cerebrum are quite rare. Only 11 authentic cases of such tumors of the spinal cord are recorded in the literature. Lindau could find only 4 reports of comparable neoplasms in the stem, while Cushing and Bailey listed 4 published cases, which they felt fairly confident were instances of cerebral hemangioblastomas. Lindau claimed that they did not occur in the cerebrum. Recently, Rochat and Keller each described a case of a cerebral hemangioblastoma.

Roman's publication (1913) was the first reliable report of a spinal cord case. A single lesion was found in the thoracic cord. This was similar in most respects to the growths described in our case. Koch (1913) recorded during the same year multiple angiomatous tumors of the thoracic cord. Pinner (1914) shortly thereafter described a capillary hemangioblastoma which ran the entire length of the cervical cord. Tannenberg (1924), a decade later, recorded 2 cases. In 1 of these a vascular tumor was present in the upper lumbar cord and similar lesions in two nearby spinal ganglia, while the 2nd case showed a single specimen of the same type of growth in the lower portion of the lumbar cord. Schuback (1927) described a large capillary hemangioma of the thoracic cord. Kernohan, Woltman and Adson (1931) in a report of 51 cases of intramedullary tumors of the spinal cord mentioned four hemangioblastomas, two being present in a single case. No details as to location within the cord were given. The histological description was quite characteristic. The high percentage of angiomatous tumors in this series is unusual and does not correspond to the small percentage of such verified tumors in the literature. It may be purely coincidental that the hemangioblastomas bulk so large in the report of Kernohan *et al.* Russell (1932) described a capillary hemangioma of the cervical

cord. In the same year Guillaín, Bertrand and Lereboullet recorded a case of multiple hemangioblastomas of the central nervous system.

The accompanying table gives some of the relevant data in these cases. Seven had single cord lesions and 5 multiple. The tumors occurred in all portions of the cord but showed some predilection for the cervical and lumbar enlargements. They occurred in the nerve roots and cauda equina as well. In all cases the tumors lay in the dorsal half of the cord, usually in the posterior columns, and showed a relation to the posterior septum. The neoplasm in the cervical portion in the case recorded here illustrates this point well. The growth in the posterior horn in the lower thoracic cord did not appear to have any relation to either the posterior septum or the pia. Four of the 12 cases showed an association of the cord lesions with cerebellar hemangioblastomas. The cerebellum is the favorite site for these neoplasms. It is interesting to note that 5 of these cases showed the associated lesions of Lindau's syndrome. Three of the cases revealed angiomatous growths in the stem. Each had a tumor in the medulla and 1 a tumor in the midbrain as well. There was a single record of concomitant involvement of the cerebrum.

Cushing and Bailey clearly differentiated between the angiomas and hemangioblastomas of the central nervous system in their monograph on blood vessel tumors of the brain. The angiomas they classified as malformations composed of arteries and veins, stressing the point that nervous tissue in various states of preservation was always found between the vessels of these lesions. The hemangioblastomas they considered to be true vascular neoplasms. In addition, a mass of dilated capillaries with nervous parenchyma between the vessels or single dilated tortuous arteries or veins were separately grouped as telangiectases.

The hemangioblastomas of the central nervous system appear to be true neoplasms, capable of growth which is sometimes fairly rapid. The analogous tumors of the retina can be directly observed in their growth. They do not metastasize nor are they apparently apt to spread along the ventricular system or subarachnoid space. A number of things point to a congenital factor in the development of these neoplasms. As Sabin has shown, the vascular network is laid down by mesodermal elements *in situ*, and it seems not unlikely that some maladjustment in this genesis may leave a nucleus for such a tumor. As Lindau has pointed out, the most common location

for the hemangioblastomas is in the rhombencephalon. The work of Karlefors has shown that in the third fetal month a vascular mesenchyme is found lying upon the posterior medullary velum. This is the anlage for the local meninges, the vascular plexus of the choroid of the fourth ventricle and of the vascular network in the areae postremae of the medulla. It is only toward the end of the third fetal month that the cerebellar hemispheres begin to develop. Lindau suggests that a portion of this original vascular plate lying in the midline might be pinched off and carried laterally into the developing hemispheres. This would account for the frequent location of the hemangioblastomas in the superficial and posterolateral portions of the cerebellum. The midline lesions and those in the medulla, which are always in its caudal portion, would be explained by the original position of the vascular plate.

As noted above, the hemangioblastomas of the spinal cord always occur in its dorsal half, very often in relation to the posterior septum. Bielschowsky, in discussing a teratoma of the cord, pointed out the possibility that portions of the surface membrane might be drawn down into the nervous parenchyma during closure of the medullary groove. This might later give rise to mesodermal growths. Tannenberg expressed a similar opinion in relation to a case of angioma of the cord.

The fact that the hemangioblastomas are often multiple and occasionally linked in Lindau's syndrome lends further support to the impression that there is a congenital factor involved in their origin.

The cerebellar hemangioblastomas may be cystic, solid, or partially cystic. The 11 cases reported by Cushing and Bailey were about evenly divided between the three types. Lindau gathered the reports of 24 cystic hemangioblastomas from the literature and added 16 of his own. The fact that he was specifically studying cerebellar cysts, however, resulted in a narrowing of the field, which led to the impression that cerebellar hemangioblastomas were almost exclusively cystic. Although predominantly so, they are not wholly of this nature. The spinal cord hemangioblastomas are all solid, although some are associated with syringomyelia, as seen in the table. We will revert to this point later in discussing the syringomyelia in our case. Lindau expressed the opinion that the cysts, both within the tumor and about it, were occasioned by circulatory disturbances in the growth giving rise to a plasmatic transudate. He

felt that the paucity of lymph channels in the nervous parenchyma did not permit ready resorption of this fluid and so cysts were formed. He ventured that the same was true of his series of cystic vascular gliomas. Cushing and Bailey, however, pointed out that extensive cystic formation was equally characteristic of the relatively non-vascular gliomas, particularly the fibrillary astrocytomas, so that the transudate cannot be attributed merely to the vascularity of the lesion. That the cyst fluid is a transudate and not simply due to degeneration of the tumor proper is evidenced by the rapidity with which such cysts refill after tapping, and the chemical resemblance of the fluid to plasma. Cushing and Bailey call attention to the fact that both angiomatous and gliomatous cysts usually have a small mural nodule of tumor such as was present in the cerebellar lesion of the case reported here. That the cystic fluid probably originates in these nodules is evidenced by the failure of such cysts to refill when the mural nodule has been removed. The causes of this cyst formation are, however, not clearly established. Of two similar hemangioblastomas of the cerebellum in Koch's case one was cystic and the other not.

Histologically the cervical hemangioblastoma in the case described here is quite characteristic of such tumors. It is predominantly capillary, although there are a considerable number of intervascular cells. Some of the reported hemangioblastomas present purely capillary zones in which no such intervening cells are present. Others are quite cellular. There appears to be an intimate relation between these interstitial cells and the lining of the capillaries, and Cushing and Bailey suggest that they arise by endothelial hyperplasia. The lipid content of many of the intervascular elements, which was so striking in the cervical and cauda equina tumors in our case, has been variously explained. Tannenberg was of the opinion that circulatory disturbances reduced the nutrition of the tumor cells so that they underwent a degenerative, fatty metamorphosis. Lindau, however, pointed out that the tumor cells stained well and did not appear degenerated. He suggested that in the formation of a cyst in the neighborhood of a hemangioblastoma there must be a slow destruction of nervous tissue. The lipoids thus liberated are taken up by the endothelial elements in the tumor. The myelomalacic area about the cervical cord tumor in the case described here might be the source of the lipid material in the solid tumors. To

TABLE I

Hemangioblastomas of Spinal Cord

Cases listed by authors	Single or multiple in cord	Location in cord	Coincident hemangioblastomas of			Accompanied by syringomyelia	Associated with Lindau's syndrome
			cerebellum	stem	cerebrum		
Roman	Single	Thoracic cord
Koch	Multiple(2)	Cervical and thoracic cord	+ (2) One in each hemisphere	+
Pinner	Single	Cervical cord	+ Cervical to upper lumbar
Tannenbergl	Multiple(3)	Lumbar cord (1) Adjacent spinal ganglia (2)	+ (2)	+ (1) Medulla	+ Upper cervical	+
Tannerbergl	Single	Lumbar cord	+ From cervical through lumbar
Schuback	Single	Thoracic cord	+ (1) Fourth ventricle through medulla to C-2	+ Almost entire cord	+
Kernohan <i>et al.</i> I	Single	Not given
Kernohan <i>et al.</i> II	Single	Not given
Kernohan <i>et al.</i> III	Multiple(2)	Not given	+	+
Russell	Single	Cervical cord	+ Cervical through thoracic
Guillain <i>et al.</i>	Multiple	Cauda equina and about lumbosacral cord	+ Multiple	+ Multiple	+ Right ventricle
Wolf and Wilems	Multiple	Cervical and lumbar cord and cauda equina	+	+ Cervical through lumbar	+

support his opinion Lindau pointed to the phagocytosing power of cells of endothelial origin. Similar tumors elsewhere in the body where lipoids are not so abundantly liberated in degenerative processes, as in the nervous system, do not show these large lipoid-containing cells. The occasional mitoses and small multinucleated giant cells encountered in the hemangioblastomas give evidence of their active growth. The reticulin network outlining the capillaries and forming intercapillary bridges is quite characteristic. Occasionally the intervacular cells become so prominent in a hemangioblastoma that they dominate the picture, although transitions can be found to capillary areas. Again the capillaries may become dilated so that they form large irregular sinuses. The latter condition was present to some degree in our cerebellar tumor. On the basis of these variations Cushing and Bailey classified the hemangioblastomas as predominantly capillary, cellular or cavernous. They described two cellular hemangioblastomas of the cerebellum and referred to another of the retina reported by Brandt. These tumors have many areas in which big epithelioid, polygonal, cuboidal and columnar cells are gathered in large clusters and columns. These cells have no processes or fibrils. Many contain considerable amounts of lipoid. This type of hemangioblastoma, however, is evidently quite rare. The capillary type with varying numbers of intervacular cells is the common variety.

From the table it may be seen that 5 of the 12 cases of spinal cord hemangioblastomas were associated with Lindau's syndrome. Beside the angiomas of the central nervous system in our case there were, as noted above, congenital cysts of the pancreas and kidneys, a benign hypernephroma of the left kidney, an adrenal rest in a retroperitoneal lymph node, and a paraganglioma of the left adrenal. As has been stated, it is extremely likely that retinal angiomas were present in this case, although we lack anatomical confirmation of this point.

Brandt, in his study of angiomas of the retinae, or von Hippel's disease, gives the average age of onset of symptoms as 25 years, with some cases beginning in childhood but none apparently after 45. The average age of onset of symptoms in angiomas of the cerebellum, as given by Lindau, is 32 years. When both are present in Lindau's syndrome the angiomas of the retinae is the first to give symptoms, while the central nervous system lesions are not in evi-

dence until a good deal later. In the case recorded here the eye disturbances began at the age of 21, while the cerebellar and cord symptoms did not appear until the patient was 32.

Lindau points out a familial disposition to angiomas of the retinae and similarly of Lindau's syndrome. Kufs has gathered data on this point and calls attention to the fact that unaffected members of the affected family may transmit the disease.

In the case described here the father had died of brain tumor at the age of 36 and the mother of "spinal meningitis" at 28 years of age. A twin brother and sister had died at birth. Although no details are known as to the type of tumor which the father had, it is possible that it was an hemangioblastoma. It is uncertain whether the conditions in the other three were related.

Angiomas of the retinae has been described twice as frequently in men as in women, while angiomas of the central nervous system occurs with about equal frequency in the two sexes.

Lindau considers a cystic pancreas one of the most constant features of his syndrome. Very often the cysts may permeate the entire pancreas and in 2 of the recorded cases a glycosuria was present. These pancreatic cysts are lined by a single layer of cuboidal cells which may become flattened and endothelial-like, as was true in our case. Some cysts are lined by columnar epithelium and others show papillary proliferation. Both Berblinger and Lindau consider them to be dysontogenetic in origin. According to Lindau the work of Siwe shows that in the second and third fetal months the branching anlage of the pancreas and a vascular mesenchyme from the dorsal mesentery intertwine in growth. A disturbance in the equilibrium between the mesodermal and epithelial tissues might lead to the snaring-off of clusters of epithelial cells from which the cysts could later develop.

Next in frequency, as concomitant lesions in his syndrome, Lindau places the malformations and tumors of the kidneys and adrenals. The cystic lesions of the kidneys in our case are undoubtedly congenital in origin and similar cysts were described in many other cases of this disease, 10 of the original 15 gathered by Lindau having them. Hypernephromas were present in 6 of these 15 cases and such a tumor was present in the left kidney of our case. The adrenal rest in the lymph node is quite unusual. The benign appearance of these nodules made it certain that they were not metastases from the

kidney and were additional congenital lesions. The paragangliomas of the adrenal in our case are the first that have been encountered in a case of Lindau's disease. They are typical examples of this uncommon type of tumor.

The familial occurrence and the presence of coincidental lesions in many organs prompted Lindau to consider the syndrome named for him as one in which there were many congenital rests and malformations referable to one of the embryonic layers. This he took to be the mesoderm and suggested that the developmental disturbance probably occurred during the third fetal month. At this time there occur vascularization of the retina, formation of the vascular mesodermal roof-plate of the fourth ventricle and ingrowth of mesodermal elements into the pancreas. Maldevelopment at this time may lead to the formation of renal cysts. He called attention to the similarity of the condition to neurofibromatosis and tuberous sclerosis. In neurofibromatosis there are multiple tumors of the peripheral and sometimes central nervous system accompanied by skin pigmentation. In tuberous sclerosis there are focal glioses of the brain, gliomas, rhabdomyoma of the heart, embryonal renal tumors and renal cysts. In both conditions there may be occasional retinal tumors.

As noted in the table of spinal hemangioblastomas, 7 of the 12 cases showed an associated syringomyelia. This is not an uncommon accompaniment of intramedullary cord tumor. Of the 51 intramedullary tumors of the cord reported by Kernohan *et al.*, 10 came to autopsy and 9 were true neoplasms, 1 being a tuberculoma. Five of these 9 cases were associated with syringomyelia. One was the hemangioblastoma included in our table, 2 were ependymomas, 1 was a medulloblastoma, and the last was an oligodendroglioma. In each of these cases the syringomyelia was described as quite characteristic. The cavities were extensive, surrounded by gliosis and not lined by ependyma.

This association of syringomyelia with spinal cord tumors has been explained in various ways. Lindau is of the opinion that these cavities in the cord are simply elongated cysts similar to the cysts so commonly encountered about cerebellar hemangioblastomas and due to transudation from the vessels of the tumor. He believes that they show only a superficial resemblance to true syringomyelia. The circulatory disturbances in vascular tumors cause edema which spreads

in the nervous parenchyma about the tumor. Edema of the intramedullary hemangioblastomas and of the surrounding parenchyma was quite definite in our case. The spread of the edema fluid occurs, as in hematomyelia, in narrow irregular columns up and down the cord, usually for long distances. Where the fluid collects cavities form, the nervous and glial tissues being compressed. The pressure of the fluid causes a reactive gliosis producing the wall of the syringomyelic cavity. It is true that the syringomyelic cavities in these cases associated with cord tumors usually course from the tumor, stretching away both above and below it. The syringomyelia of the cervical cord and the syringobulbia with which it is continuous appear to be definitely related to the hemangioblastoma in the upper cervical cord. The cavities in the lower thoracic region and lumbar cord seem equally related to the neoplasm of the lower thoracic cord. This is rather small and the cavities large. Many cystic cerebellar hemangioblastomas, however, have small mural nodules and quite a large cyst. Kirch also points out that solid gray glial columns, such as are considered to antedate the cavity in many true syringomyelias, can be found in relation to intramedullary cord tumors. This is true in Schuback's case in which such solid glial columns were found flanked by cavities. Kirch suggests that in these cases no collections of edema fluid have formed, a more diffuse infiltration of the tissue by the serous transudate having occurred. The occurrence of syringomyelia in relation to such tumors as the medulloblastoma and oligodendroglioma of the cord reported by Kernohan *et al.*, is difficult to explain on the basis of a circulatory disturbance in a vascular tumor with transudation. The same was true, as noted above, in attempting to explain the genesis of neoplastic cerebellar cysts on the same basis when one considers the cysts accompanying the relatively non-vascular fibrillary astrocytomas of the cerebellum. Kernohan *et al.*, however, in discussing the syringomyelia in their case of spinal cord hemangioblastoma were of the same opinion as Lindau and Kirch. Tannenberg also expressed the opinion that the syringomyelia in these cases is comparable to the cysts which form in relation to tumors of the central nervous system. He believes them to be due to softenings in the region of such neoplasms with resultant cavity formation and gliosis, rather than a mere transudation, fluid collection and tissue compression. He considers the necroses to be due both to circulatory disturbances locally and to

toxins elaborated by the tumor. That considerable destruction can occur about spinal cord hemangioblastomas was evidenced in the degeneration about the intramedullary tumors in our case and these degenerated zones bridge from tumor to syringomyelia.

Russell likewise believes that anemic or hemorrhagic softenings due to the presence of a tumor in the cord may give rise to the syringomyelia. She quotes Cornil and Ranvier for evidence that softening following trauma follows the same distribution as hemorrhage in the cord. A column of softened tissue in the posterior columns and horns, parallel with the long axis of the cord, may extend for long distances. Finally a cavity with a glial wall is formed. To prove that such softenings occur secondary to cord tumors she gives the details of an autopsy from her own laboratory. It was a case of cord metastasis in the first and second lumbar segments from a carcinoma of the lung. There was hemorrhagic softening of the cord to the tenth, and anemic softening to the sixth thoracic segment. Below the tumor the rest of the lumbosacral cord was involved in hemorrhagic softening.

Another explanation of the association of angiomatous neoplasms with syringomyelia is that they are coincident, multiple, congenital anomalies. Bielschowsky expressed this opinion and Jonesco-Sisesti more recently was strongly in its favor. Both adduce the evidence of the conjunction of teratomas and teratoid tumors of the cord with syringomyelia. Furthermore, the occurrence of the syringomyelia with multiple congenital malformations, such as occur in Lindau's disease and in neurofibromatosis, would be in favor of this theory. Our own case and 4 others listed in the table showed this association. A number of the reported cases and 2 of those listed in the table, however, do not show such congenital abnormalities.

A final possibility is that hemorrhages from intramedullary tumors, in particular hemangioblastomas, may produce hematomyelia which results in a syringomyelia. Russell discusses this theory and points out that Ohlmacher's case of a cavernous angioma of the cervical cord was accompanied by a hematomyelia which extended for some distance above and below the tumor. She quotes a case of her own, an ependymal glioma of the lumbar cord associated with syringomyelia. There was evidence of an old hemorrhage in the borders of the syringomyelic cavity and in the walls of a cyst within the tumor. Russell believes that this may be the cause of the associ-

ation in some cases, but most often when there is hemorrhage it is secondary to the circulatory disturbance with necrosis.

In our case the syringomyelia is anatomically quite typical and in no wise distinguishable from similar lesions of this nature. Its wall resembles that of the cerebellar cyst and in a sense the syringomyelic cavities can be considered "cysts" related to the spinal cord tumors. They are intimately associated with the neoplasms, being ranged about them. The markedly edematous areas with the often intense accompanying degeneration are directly continuous with the syringomyelia, or contiguous to the tumors. They point to a local vascular disturbance with transudation.

From the evidence of our own material, therefore, we believe these cavities in the cord to be true syringomyelia and yet comparable to the similar cerebellar cysts and similar cysts elsewhere associated with tumor. The most plausible explanation of their formation is that a columnar edema and necrosis occurs secondary to circulatory disturbances produced by the tumor, with subsequent cavity formation and mural gliosis.

SUMMARY

A case of multiple hemangioblastomas of the spinal cord forming part of Lindau's syndrome is presented. These intramedullary tumors are associated with a syringomyelia and syringobulbia. The other lesions are a cystic cerebellar hemangioblastoma, congenital cysts of the pancreas and kidneys, a benign hypernephroma of the left kidney, an adrenal rest in a retroperitoneal lymph node and three paragangliomas of the left adrenal.

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DESCRIPTION OF PLATES

PLATE 132

- FIG. 1. Hemangioblastoma of cervical cord. Hematoxylin-eosin stain.
- FIG. 2. Hemangioblastoma of lower thoracic cord. Hematoxylin-eosin stain.
- FIG. 3. Syringomyelia. Lumbar cord. Phosphotungstic acid hematoxylin stain.
- FIG. 4. Syringobulbia. Lower medulla. Phosphotungstic acid hematoxylin stain.



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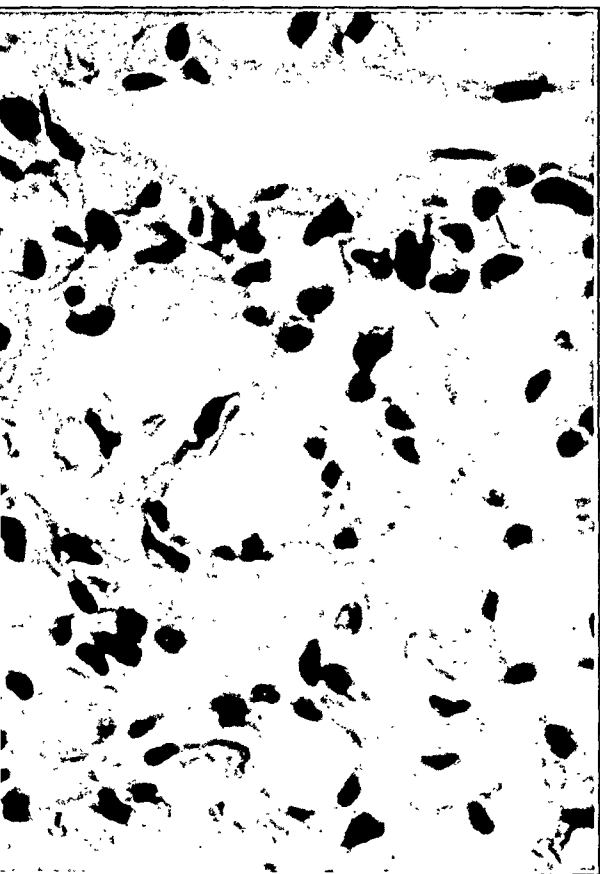
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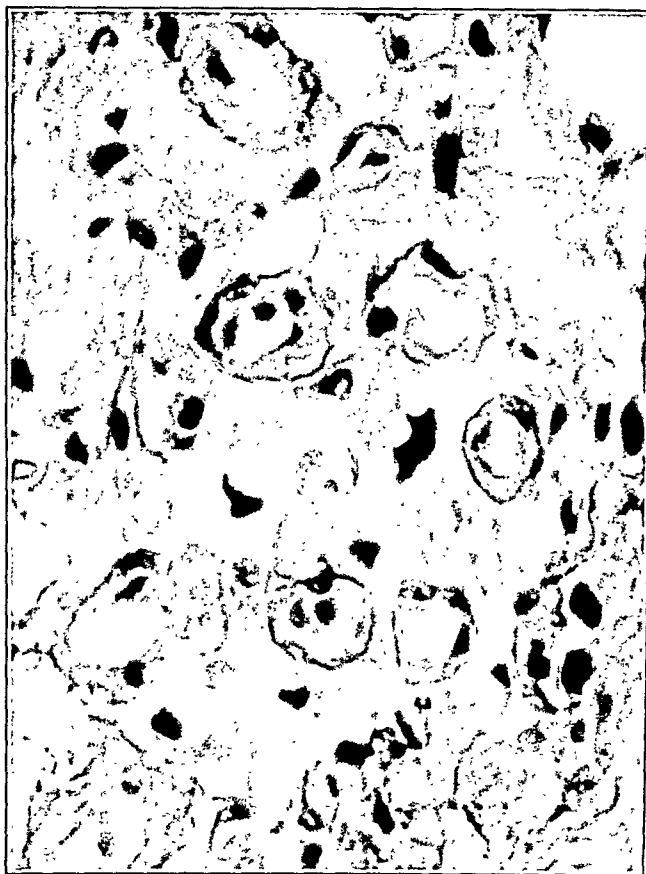
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PLATE 133

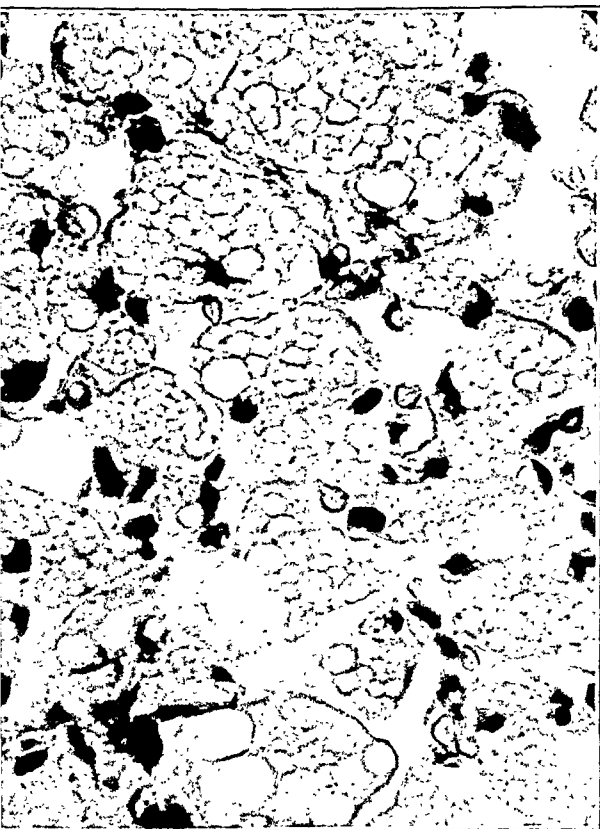
- FIG. 5. Capillary zone in cervical cord hemangioblastoma. Hematoxylin-eosin stain. $\times 720$.
- FIG. 6. Capillary zone in thoracic cord hemangioblastoma. Hematoxylin-eosin and Laidlaw stains combined. $\times 450$.
- FIG. 7. Pseudoxanthomatous cells between the capillaries in a cellular area of the cervical cord hemangioblastoma. Hematoxylin-eosin stain. $\times 720$.
- FIG. 8. Pseudoxanthomatous cells in thoracic cord hemangioblastoma. Phosphotungstic acid hematoxylin stain. $\times 720$.



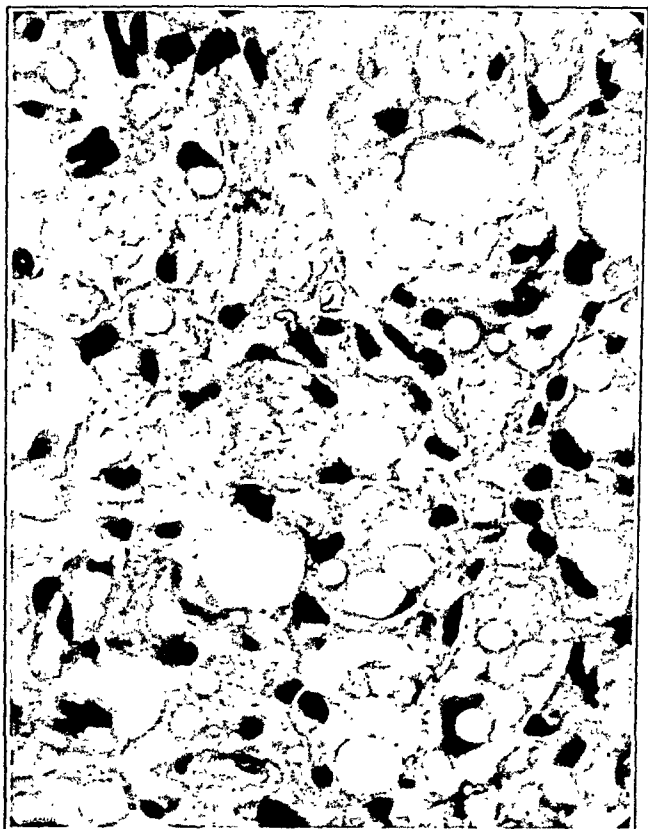
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PLATE 134

FIG. 9. Capillaries in cervical cord hemangioblastoma. Note tendency to radiate from the large, thick-walled channels. Laidlaw stain. $\times 80$.

FIG. 10. Benign hypernephroma of the left kidney. Hematoxylin-eosin stain. $\times 100$.

FIG. 11. Adrenal rest in retroperitoneal lymph node. Hematoxylin-eosin stain. $\times 100$.

FIG. 12. Cysts in pancreas. Hematoxylin-eosin stain. $\times 80$.



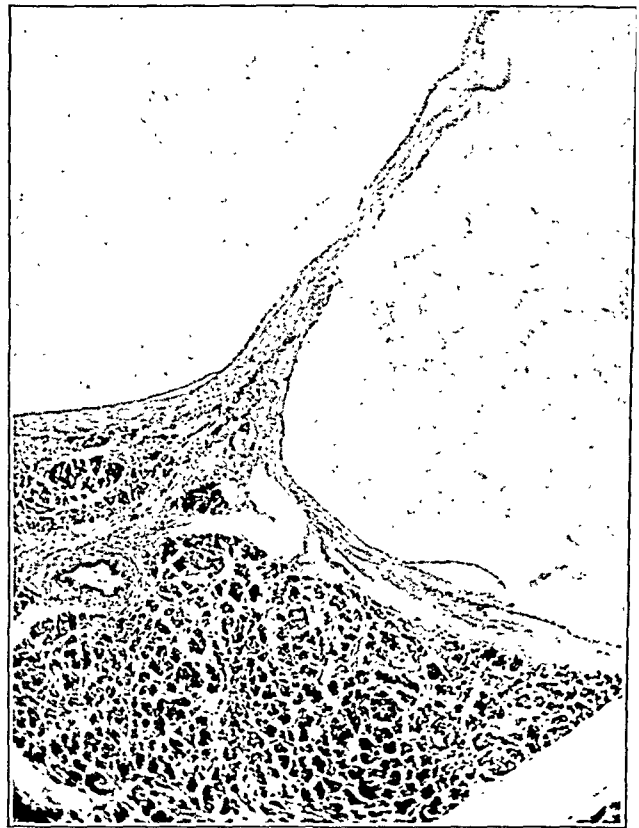
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THE AMERICAN JOURNAL OF PATHOLOGY

VOLUME X

SEPTEMBER, 1934

NUMBER 5

FORMATION OF INTERCELLULAR SUBSTANCE BY THE ADMINISTRATION OF ASCORBIC ACID (VITAMIN C) IN EXPERIMENTAL SCORBUTUS *

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In 1919 Aschoff and Koch ¹ expressed the belief that in scurvy the primary deficiency consisted of a lack or faulty development of cement substance. They inferred that the condition at the costochondral junction in human scorbutic material was due to the inability of osteoblasts to form osteoid tissue. The experiments of Wolbach and Howe ² demonstrated without any doubt that the immediate effect of orange juice administration in the repair of scorbutus is prompt deposition of intercellular material. This reparative process was clearly shown in several ways, notably by the renewed formation of dentine in the incisor teeth of guinea pigs, the deposition of a homogeneous matrix by the periosteal layer of cells and, finally, by the formation of osteoid and osseous trabeculae in the *Gerüstmark* of the costochondral junctions. More recently Wolbach ³ demonstrated that the deposition of collagen in the organization of blood clots in the state of absolute scorbutus was referable to the administration of vitamin C and that it represented the product of fibroblastic secretory activity. The earlier studies of Wolbach and Howe led these investigators to consider scorbutus as a state characterized primarily by a cessation in the normal formation of intercellular substance on the part of supporting tissues.

* An abstract of this study was read before the American Society for Experimental Pathology in New York City, March 30, 1934.

Received for publication June 4, 1934.

The object of this brief report is to record the histological response produced by the administration of crystalline ascorbic acid (vitamin C) in the amelioration of experimental scorbutus in guinea pigs. That ascorbic acid prevents the development of scorbutus in guinea pigs and cures the condition when established has been proved. The observations that are to follow show that the histological repair is identical with that produced by vitamin C in orange juice.

Advances toward the isolation of vitamin C in its crystalline form have been rapid since 1928 when Szent-Györgyi isolated a reducing substance from various plants and subsequently from adrenal cortex which he called *hexuronic acid*.⁴ From the very beginning Szent-Györgyi recognized the similarity of this acid to vitamin C. In the early part of 1932, in collaboration with Svírbely, this investigator reported that a daily allowance of 1 mg. of hexuronic acid afforded complete protection against scurvy in a 90 day test.⁵ According to Szent-Györgyi this substance is a monocarboxy acid corresponding to the formula $C_6H_8O_6$. More recent studies in association with Haworth⁶ have shown that it has evidently no relation to uronic acid and that it is most likely a lactone. Szent-Györgyi proposed the name of *ascorbic acid* for the crystalline product. Independently, and practically simultaneously with the isolation of ascorbic acid by Szent-Györgyi, King and his collaborators obtained crystals from lemon juice that had identical effects on scorbutic animals.⁷ Recently Reichstein and his collaborators⁸ succeeded in synthesizing a product from l-xylosone having properties that they believe are identical with those of natural ascorbic acid.

The crystalline vitamin C preparation, as isolated and used in the experiments that are to be reported here, was obtained from lemon juice by modifying only slightly the original methods of extraction, as reported in the various communications of King and his co-workers.⁹⁻¹² The reader is referred to these publications for details of the chemical technique. There is, however, one point that perhaps deserves mentioning. In the final step of extraction crystallization of ascorbic acid was found more effective when the material was maintained at minus 20° C for several days. Furthermore, its potency seems to be distinctly decreased when the various steps in the isolation process are carried out in the presence of oxygen. One such preparation, when administered to scorbutic guinea pigs, yielded definite reparative response in the incisor teeth but failed to amelio-

rate the condition in the *Gerüstmark* of the costochondral junction. This would indicate that the reparative response to antiscorbutics is more sensitive in the incisor teeth than at the costochondral junction. A second preparation in which adequate precautions were taken to isolate the material in a relatively oxygen-free atmosphere by bubbling CO₂ during the various evaporations *in vacuo* yielded a product sufficiently potent so that adequate repair could be demonstrated both at the costochondral junction and in the incisor teeth.*

The diet employed was the same that Wolbach and Howe had used in 1926. The guinea pigs had an initial weight ranging between 250 and 400 gm. The ascorbic acid solution was administered either orally or parenterally, 3 to 5 mg. per day as a rule. The treatment was started 2 to 3 weeks following the onset of the scorbutic diet, at a time when the symptoms of the disease were conspicuous. The treatment with ascorbic acid was continued for a period varying from 2 to 15 days, after which the animal was chloroformed and a thorough postmortem examination performed.

The histological studies were centered chiefly in following the reparative processes occurring in sections of the costochondral junctions and through the incisor teeth. Wolbach and Howe in their original study found that these structures gave them exceedingly good material for studying scorbutic sequences. In brief, they have pointed out that the important change in the incisors of scorbutic guinea pigs is cessation of formation of dentine and separation of the odontoblast layer from the dentine (Fig. 1). In complete scorbutus the presence of liquid separating the odontoblasts from the dentine seems to be the result of a defective secretion of the odontoblasts. Furthermore, they showed that the administration of orange juice is rapidly followed by the deposition of newly formed dentine in the irregular contours of the odontoblast layer. On the basis of their findings they expressed the view that "the missing factor or agent which the antiscorbutic enables the odontoblasts to supply is evidently one effecting the jelling or setting." At the costochondral junction of scorbutic animals they found that osteoblastic proliferation continues uninterruptedly without, however, producing any bone matrix. These cells assume the shape of fibroblasts, and the

* Professor Szent-Györgyi was kind enough to send us a sample of ascorbic acid which we have also been able to test on some of our scorbutic animals. The histological findings were similar to those obtained with the product isolated in this laboratory.

resulting edematous appearance of the loosely textured connective tissue has been given the name *Gerüstmark*. The initial administration of orange juice is promptly followed by a diffuse deposition of osteoid matrix. On further treatment with the antiscorbutic Wolbach and Howe described the formation of definite osteoid and osseous trabeculae replacing the loosely textured *Gerüstmark*. Here again, as in the case of the odontoblasts, they interpret the activity of the osteoblasts in scorbutus as that of cells producing a defective liquid product.

RESULTS

The data obtained following the administration of ascorbic acid to scorbutic guinea pigs were exactly in accord with the previous findings of Wolbach and Howe with orange juice. This would seem to prove that the deposition of intercellular substance subsequent to the feeding of orange juice is due to vitamin C *per se* and not to some other ingredient. Most of the animals showed distinct symptomatic improvement soon after the ascorbic acid feeding was initiated, as indicated by both increased weight and restoration of appetite.

The incisors of scorbutic guinea pigs receiving no ascorbic acid showed, as stated above, the characteristic separation of dentine from the odontoblast layer and a somewhat shrunken pulp (Fig. 1). Scorbutic guinea pigs given the crystalline product displayed marked deposition of dentine precisely as had been previously found with orange juice (Fig. 2). Furthermore, the amount of intercellular dentine laid down follows roughly the total amount of ascorbic acid administered. Parenteral injection of the crystalline product is at least as effective in producing reparative reactions as oral administration.

Sections through the costochondral junction of scorbutic animals show the typical loosely textured *Gerüstmark* containing fibroblast-like cells producing no osteoid matrix. There is considerable hemorrhage; the periosteum is thinned out with cell bodies in close apposition to each other and devoid of the normal homogeneous matrix (Fig. 3). By contrast Figure 4 illustrates the same region in a scorbutic guinea pig that received one intraperitoneal and three daily subcutaneous injections each of 5 mg. of ascorbic acid. The site of parenteral injection was shifted from day to day so as to obviate any retardation in absorption by the possible development of an inflammatory reaction sufficiently severe to obstruct lymphatic drainage.¹³

The *Gerüstmark* is interspersed with osteoid trabeculae extending from the columns of cartilage to the marrow. The periphery of these trabeculae is lined by numerous osteoblasts. The repair is complete, involving also cartilage and periosteum (Fig. 5). Adjacent to the cartilage columns, cells are seen that form either osteoid matrix, chondromucin or the homogeneous collagenous matrix of periosteal tissue (Fig. 5). In a guinea pig sacrificed after receiving a total of 75 mg. of the crystalline material orally over a period of 15 days the newly formed osteoid trabeculae at the *Gerüstmark* were found ossified, with evidence of progressive bone resorption (Fig. 6).

DISCUSSION

The observations cited above show that the administration of the crystalline material known as *ascorbic acid* to scorbutic guinea pigs induces reparative reactions identical in character with those obtained with orange juice. The cessation in the formation of intercellular substance by supporting tissues in experimental scorbutus is therefore doubtless due to the lack of vitamin C, which is ascorbic acid. It is interesting to note that a function of cells as important as the production of intercellular matrix seems to depend on the presence of a relatively simple chemical substance, having as its empirical formula $C_6H_8O_6$. The mechanism of this reaction still remains to be elucidated. Further studies are being conducted in an endeavor to clarify this problem. The observations of Szent-Györgyi suggest the intimate rôle that ascorbic acid may play in cellular oxidation¹⁴ in connection with its unusual reactivity as a reducing agent. This investigator advances the theory that ascorbic acid plays a central rôle in cellular reactions by acting as a regulator of potential changes, in this way preserving protoplasm against oxidation. He showed that silver salts, even in acid solution, are immediately reduced by ascorbic acid, a capacity unparalleled by substances composed solely of carbon, oxygen and hydrogen. While acting as a reducing agent ascorbic acid oxidizes itself, this being a reversible phenomenon. Szent-Györgyi expresses the belief that the biological function of ascorbic acid resides in its oxidation-reduction properties, in that it acts as a sort of buffer which links the various oxidizing enzymes participating in cellular metabolism. He has obtained some support for this view in studies on the respiration of the cabbage leaf.¹⁵ The

oxidation-reduction potential of ascorbic acid has also recently been studied by Fruton¹⁶ and others. Evidences of ascorbic acid acting as an activator of enzyme action have also recently been brought forward by Purr,¹⁷ and Karrer and Zehender.¹⁸ Harrison¹⁹ has lately studied the oxygen consumption of tissues removed from scorbutic animals and has shown that the uptake is considerably lower than that from normal animals. The addition of small amounts of ascorbic acid to such tissues *in vitro* brings about an increase in the oxygen consumption. In the case of normal tissues, however, the oxygen uptake remains unaffected by the addition of ascorbic acid. It is conceivable, in view of such observations, that the lack of intercellular substance in the supporting tissues of scorbutic animals, as evidenced histologically, may be an expression of reduced cellular oxidation resulting from a deprivation of ascorbic acid.

SUMMARY AND CONCLUSIONS

Ascorbic acid (vitamin C in crystalline form) administered orally or parenterally to scorbutic guinea pigs induces reparative processes, as demonstrated by the renewal of dentine formation in the incisor teeth and by the deposition of osteoid matrix and chondromucin at the costochondral junction. These results on the formation of intercellular substance are in accord with those previously obtained by Wolbach and Howe by feeding orange juice. They furnish additional support for the view that ascorbic acid is indistinguishable from vitamin C. The evidence obtained indicates that a relatively simple chemical substance, ascorbic acid, controls the deposition of intercellular substance. The possible mechanism involved in this reaction is discussed from the standpoint of the properties of ascorbic acid as a reducing agent in relation to cellular oxidations.

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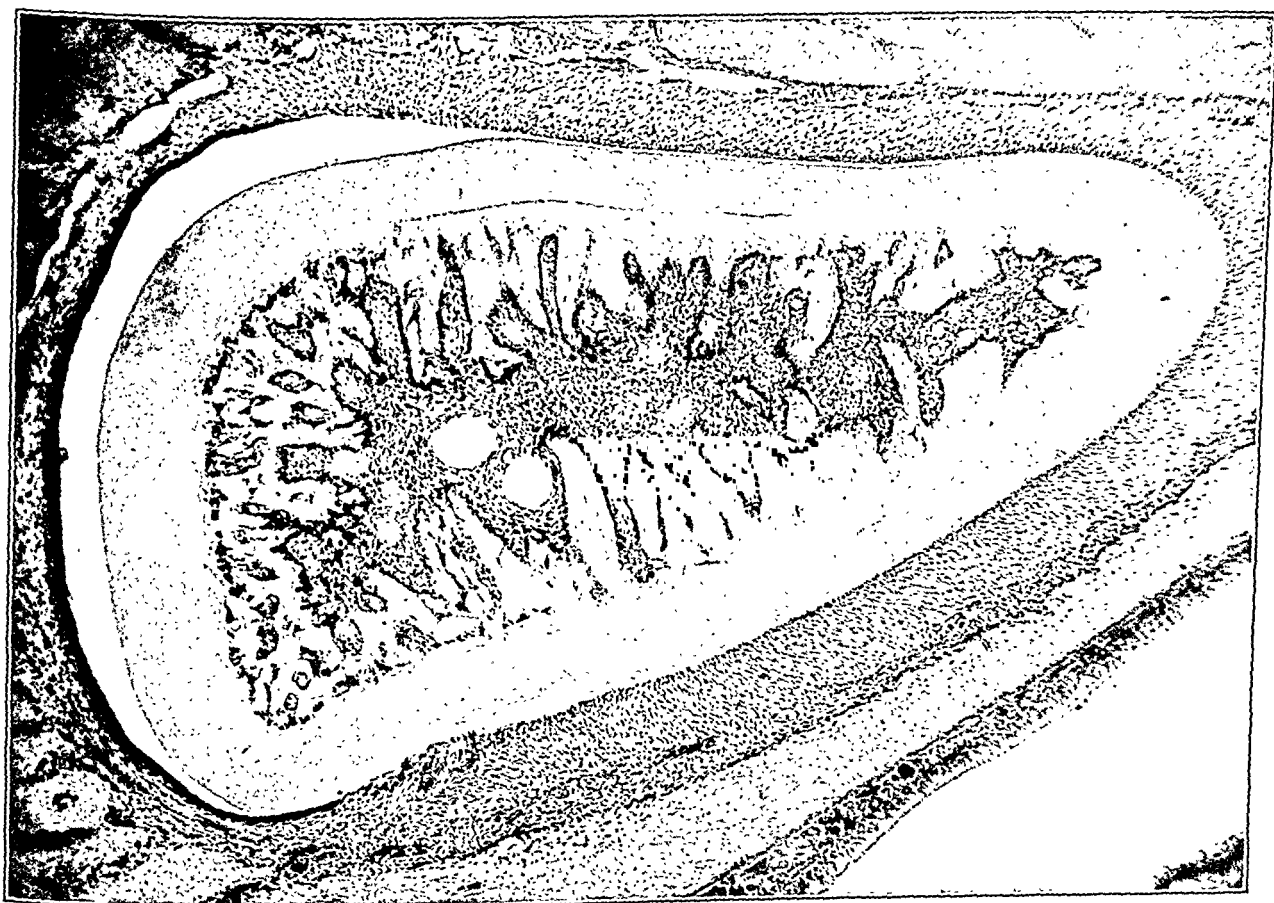
DESCRIPTION OF PLATES

PLATE 135

- FIG. 1. Section through incisor of a scorbutic guinea pig. The separation between the odontoblast layer and the dentine is striking. Low power.
- FIG. 2. Section through incisor of experimental scorbutic guinea pig. 20 mg. of ascorbic acid administered parenterally over a period of 4 days. The animal was then killed. Note the large amount of newly formed dentine. Low power.



1



2

PLATE 136

FIG. 3. High power drawing through costochondral junction of scorbutic guinea pig, showing the loosely textured *Gerüstmark*. There is some evidence of hemorrhage. Since the cells have failed to produce any intercellular substance it is practically impossible to differentiate osteoblasts from fibroblasts.

FIG. 4. Section through costochondral junction of experimental scorbutic guinea pig that had received four parenteral injections of ascorbic acid aggregating to 20 mg. Note at the *Gerüstmark* the extensive repair reaction in the form of newly deposited osteoid trabeculae. $\times 175$.



3



4

Menkin, Wolbach and Menkin

Formation of Intercellular Substance

PLATE 137

- FIG. 5. Same guinea pig as Fig. 4, showing reparative reaction of cartilage and periosteum at the costochondral junction following four parenteral injections of ascorbic acid. About $\times 160$.
- FIG. 6. Costochondral junction of experimental guinea pig that had received orally 75 mg. of ascorbic acid over a period of 15 days. Note the osseous trabeculae with progressive bone resorption. Compare with deposition of osteoid matrix in Fig. 4 following only 4 days treatment with ascorbic acid. $\times 175$.



5



6

INCLUSIONS IN RENAL EPITHELIAL CELLS FOLLOWING THE USE OF CERTAIN BISMUTH PREPARATIONS *

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It is not the purpose of this paper to discuss the pathology of bismuth nephritis in general, but rather to call attention to peculiar epithelial inclusions, which seem to be definitely related to the administration of certain bismuth compounds. The fact that these bodies are found within the nucleus as well as in the cytoplasm lends them particular interest and justifies a detailed description. The bodies were first observed in Case 1. A second case, in which a different bismuth preparation was used, and which also showed similar inclusions in the renal epithelium, is also reported.

CASE REPORTS

CASE 1. P. H. History No. 357,602. The patient, E. R., a male, 46 years of age, was admitted to the Presbyterian Hospital on Oct. 28, 1932, and again on Dec. 9, 1932. The chief complaint was epigastric pain and loss of weight for 5 months. The family history was unimportant. The patient had been healthy and robust until the onset of the present symptoms. There was a history of gonorrhea 23, 20 and 9 years ago, but no syphilitic infection. The Wassermann reaction was reported negative in 1923, 9 years previous to the present illness.

Five months ago the patient began to have pain and a feeling of fullness in the epigastrium. The pains were gnawing in character, occurred 1 to 2 hours after meals and occasionally at night. The pain was relieved by vomiting. Over a period of 3 months there was a loss of 16 pounds in weight. He entered the hospital in October, remaining for 3 weeks. Physical examination at that time disclosed a palpable mass in the epigastrium. The Wassermann reaction was 4 plus, and because of the possibility that the mass was a gumma of the liver he was referred to the Dermatological Clinic for antiluetic treatment. On November 22nd, and again on the 25th, 29th and on December 2nd he received 2 cc. of bismocymol (0.1 gm. bismuth) intramuscularly. In addition, he was given three intravenous injections of Old Salvarsan, totalling 0.6 gm., and 1 gr. of mercuric salicylate intramuscularly.

There was no decrease in the size of the abdominal mass under treatment. The weakness and loss of weight progressed, and the patient was readmitted to the surgical service.

Physical Examination: The temperature was 98.4, pulse 80, respiration 20, blood pressure 115/90. The head and thorax were normal. In the epigastrium

* Received for publication November 2, 1933.

a large, hard, irregular mass could be felt with the lightest touch and its outlines could be seen through the abdominal wall. It extended two-thirds of the way to the umbilicus and was slightly tender. Cervical, axillary and right epitrochlear glands were definitely large but not tender. Examination otherwise negative.

Laboratory Findings: Hemoglobin 75 per cent, red blood cell count 4,150,000. Wassermann reaction 4 plus. Stool guaiac-negative. X-ray showed a broad crater shadow along the lesser curvature of the stomach. Urinalysis showed a trace of albumin, many hyaline and granular casts and occasional white blood cells.

An exploratory laparotomy was performed 3 days after admission and an extensive carcinomatous infiltration of the liver was found. This was confirmed by examination of an excised specimen. Following the operation a hematoma occurred in the lower third of the wound. The patient became increasingly weak and died on the 12th day after operation.

Clinical Diagnoses: Carcinoma of stomach with metastases to liver; syphilis.

Postmortem Examination

Autopsy No. 11,124, performed 16 hours after death.

Anatomical Diagnoses: Gastric ulcer and carcinoma, metastases in regional lymph nodes, liver and lung; syphilitic aortitis, syphilitic orchitis with gummas; atherosclerosis.

The stomach was the seat of a carcinomatous ulcer 2.5 by 3 cm., situated on the lesser curvature 4 cm. from the pylorus. Nodules of tumor tissue were seen in the base of the ulcer and in the lesser omentum. The liver weighed 5350 gm., was greatly enlarged and filled with carcinomatous nodules. Normal liver tissue was found only in one small area in the right lobe. The aorta presented gross and microscopic lesions of syphilitic aortitis. Both testes were fibrotic and contained gummatous areas of caseous necrosis.

Kidneys: No gross changes were visible. The right kidney weighed 140 gm., the capsule was easily removed, the surface smooth and brownish. The cortex was 7 mm. wide. The markings were regular and distinct. There was no gross vascular thickening. Pelves and ureters were normal. The left kidney was similar in appearance.

Microscopic Examination of Kidneys

Microscopically no significant changes are found in the glomeruli. The capillaries contain a moderate amount of blood, the basement membrane is not thickened and there is no increased cellular content. A small amount of granular coagulum is seen in some of the capsular spaces.

Interesting changes, however, are found in the epithelial cells of the convoluted tubules, and chiefly in the distal portions. The lining cells are atypical, often flattened or irregular in shape; the nuclei are variable in size and occasional mitotic figures are seen. Often the lumen is filled with exfoliated necrotic cells or cellular detritus which stains deeply with eosin. In some tubules one has the impression of a coagulative necrosis affecting the portion of the cell contiguous to the lumen, but leaving the basal portion of the cell still viable.

Peculiar bodies are found in many of these atypical or degenerating epithelial cells. They are both cytoplasmic and intranuclear. Their size varies from 2 to 5 microns, averaging perhaps half the size of a red blood corpuscle. Their shape is usually spherical, although elliptical and obovate forms are occasionally seen. They have, in unstained formalin-fixed frozen sections, a slightly brownish color, and are highly refractile with a dark, singly contoured membrane. In some tubules they are quite numerous; others contain only one or two.

Intranuclear bodies are usually surrounded by a pale halo (Fig. 1). The nucleus containing the inclusion is often slightly enlarged or hydropic and the nucleolus displaced to one side against the nuclear membrane. One nucleus is found in which the slightly elongated inclusion has apparently bulged out the nuclear membrane preparatory to escaping into the cytoplasm (Fig. 2).

The cytoplasmic bodies are usually located between the nucleus and free border, or at one side of the nucleus (Figs. 3 and 4). Frequently they are found within the necrotic desquamated cells within the lumen. In general, their presence is associated with, possibly leads to, necrosis of the containing cell. A characteristic inclusion, however, is found within a cell undergoing mitosis, and many of the cells with intranuclear bodies show little if any degenerative change. This suggests that the inclusions are first formed within the nucleus, to be later extruded as the cell degenerates.

CASE 2. P. H. History No. 405,156. The patient, a female, 60 years of age, was first admitted to the Neurological Institute on Dec. 11, 1933. For 3 years she had suffered from generalized headaches, attacks of flushing and dizziness. A year ago she had a slight stroke, with speech impairment and right hemiparesis. A second similar attack occurred in November, 1933.

On physical examination right hemiparesis, adiadokocinesis, rambling speech and exaggeration of reflexes on the right side were present.

Laboratory Findings: Red blood cells 4,300,000, white blood cells 7500, polymorphonuclears 70 per cent, hemoglobin 78 per cent. Blood Wassermann reaction 4 plus. Spinal fluid 0, gold curve 1111000000, Wassermann negative. Urine showed a faint trace of albumin and clumps of pus cells.

A diagnosis of meningovascular syphilis with thrombosis of left anterior and middle cerebral arteries was made. The patient was given a course of anti-syphilitic treatment. She received 10 intramuscular injections of potassium bismuth tartrate with butyn (Abbott Laboratories) over a period of 31 days, beginning December 19th. The total amount administered was the equivalent of 1 gm. of metallic bismuth.

On January 18th she developed what was thought to be pneumonia, and was transferred to the Presbyterian Hospital. The temperature was 103.2. The white blood count was 40,750, polymorphonuclears 89 per cent. The blood urea was 1.5 gm. per liter. The physical signs of pneumonia improved but several bed sores developed. A large calculus in the gall-bladder was demonstrated by X-ray. The urine contained considerable amounts of albumin and pus, and the left kidney was found enlarged. After 2 weeks Cheyne-Stokes respiration appeared and death occurred, apparently in uremia. Twenty-four hours before death the right leg became purplish, cold and edematous.

Postmortem Examination

Autopsy No. 11,469, performed 14 hours after death.

Anatomical Diagnoses: Generalized arteriosclerosis; thrombosis of arteries of right leg; gangrene of right leg; early confluent lobular pneumonia; acute tracheobronchitis; suppurative pyelonephritis and pyonephrosis, left; syphilitic aortitis; chronic cholecystitis and cholelithiasis; melanosis of colon; old salpingo-oophorectomy, left; pelvic adhesions.

Permission to examine the brain could not be obtained.

Kidneys: The left formed a sac-like mass 15 by 10 by 8 cm., weighing 110 gm. The capsule stripped easily and the surface was finely granular. The pelvis was distended with semipurulent fluid and there were several large abscesses in the substance of the kidney. The right kidney weighed 200 gm., was soft, flabby and the cortical markings were blurred, but there were no abscesses.

Microscopic Examination of Kidneys

Microscopically the left kidney shows a diffuse pyelonephritis, with the presence of numerous, short Gram-negative bacilli. The right kidney is almost free from acute suppurative changes. Autolysis is fairly marked but there appears to be little degeneration and no necrosis of the epithelial cells. Many of them, however, are

exfoliated and the lumens of the tubules are often filled with granular coagulum. There are no significant glomerular lesions and the arteriosclerotic lesions of the larger renal branches are not extreme.

The interesting feature of the kidney sections is the presence of numerous spherical globules, identical in location, size and appearance with those described in the previous case. They are found within the nuclei, but more abundantly in the cytoplasm of the epithelium of the convoluted tubules, or free in the lumen (Figs. 5 and 6).

STAINING REACTIONS AND MICROCHEMICAL TESTS OF REFRACTILE BODIES

1. With hematoxylin-eosin, after formalin or Zenker fixation, the refractile bodies are unstained but have a slight brownish tinge. They are, as has been stated, very refractile and sharply contoured.
2. With eosin-methylene blue the majority of the bodies, both intra- and extranuclear, stain intensely with the methylene blue. A few of the larger ones, however, retain their brownish tinge.
3. With Pappenheim's methyl green-pyronin they retain their brownish color, in contrast to the red-staining nucleoli, from which they can readily be distinguished.
4. They are unstained with scharlach R in frozen sections of formalin-fixed material. There are only occasional small fat droplets within the degenerating epithelial cells. The globules are not doubly refractive.
5. With Nile blue sulphate a faint greenish blue staining of the globules is obtained.
6. With 1 per cent osmic acid on frozen sections of formalin-fixed material, after washing in several changes of distilled water, a slight darkening of the globules is noted. They are not blackened, however.
7. With Spielmeyer's myelin stain intense blackening of both the intranuclear and cytoplasmic bodies is produced. The bodies are brilliantly and selectively brought out by this method (Fig. 7).
8. Ciaccio's method shows the presence of amorphous sudanophile masses in the cytoplasm of some of the epithelial cells. The globules are unstained.
9. After 10 days digestion of small slices of formalinized tissue with Merck's pancreatin the globules are unaffected, save that they show a tendency to stain with hematoxylin (Fig. 7).

10. The iron reaction with potassium ferrocyanide and hydrochloric acid is negative.

11. They are not dissolved by strong ammonia or by 20 per cent nitric acid.

12. Von Kossa's stain for calcium is negative.

The following microchemical tests for bismuth were applied.

1. With hydrosulphuric acid the bodies are slightly darker than in the unstained control sections.

2. Ammonium sulphide also produces darkening, but no diffuse black coloration.

3. With stannous chloride-sodium hydroxide the inclusions stain brownish black. Under the oil immersion minute black granules in active brownian motion are seen floating in a colorless menstruum.

4. With potassium iodide-sulphuric acid the bodies take a slightly more yellowish tinge than the unstained control.

5. Frozen sections treated according to the method of Komaya¹ with quinine sulphate and potassium iodide fail to give the characteristic bismuth reaction, although a deep brick red color was obtained with bismocymol on filter paper.

In addition to the refractive globules above described there is in Case 1 a considerable amount of yellowish brown pigment within the epithelial cells at the junction of pyramid and cortex. This is in the form of irregular, varying sized clumps. It does not blacken with hydrosulphuric acid or ammonium sulphide, nor does it give an iron reaction with potassium ferrocyanide and hydrochloric acid. It fails also to react with Komaya's reagent and the other microchemical tests for bismuth. It is not removed by the lipoid solvents used in dehydration and clearing, nor does it give a fat stain with scharlach R.

CHEMICAL DETERMINATION OF BISMUTH IN KIDNEYS

In view of the fact that the patient received intramuscular injections of bismuth it seemed of interest to determine the amount of bismuth still present in the renal tissues in each case.

A colorimetric determination was carried out on duplicate samples of formalin-fixed tissue, according to the method of Leonard.² The analysis of Case 1 gave the following results.

	SAMPLE A	SAMPLE B
Wet weight	8.2058 gm.	4.3730 gm.
Dry weight	1.9527 gm.	1.0368 gm.
Bismuth found	1.36 mg.	0.0727 mg.
Bismuth per 100 gm. wet tissue	16.57 mg.	16.62 mg.
Bismuth per 100 gm. dry tissue	69.7 mg.	69.8 mg.

Taking the combined weight of the two kidneys as 280 gm., the calculated total amount of bismuth retained by the renal tissue may be estimated as approximately 46.5 mg., or well over 10 per cent of the total amount injected. To what extent the extreme carcinomatous replacement of the liver tissue interfered with the normal storage of the bismuth in the reticuloendothelial cells (Komaya) of this organ, thus intensifying the toxic effect upon the kidney, must remain problematical. The finding of a considerable amount of bismuth in the kidney made it logical to ascribe the renal damage to this substance. Only a trace of mercury could be recovered.

An analysis of the kidneys of Case 2, carried out with the same technique, gave the following results.

Wet weight	4.6955 gm.
Dry weight	1.1016 gm.
Bismuth found	1.120 mg.
Bismuth per 100 gm. wet tissue	23.8 mg.
Bismuth per 100 gm. dry tissue	101.67 mg.
Total weight of both kidneys	310.0 gm.
Total bismuth content	73.8 mg.
Total amount injected	1.0 mg.

The kidney at the time of death therefore contained 7.38 per cent of the amount injected.

The nature of the refractile bodies within the epithelial cells still remained a problem. It seemed of interest to determine whether they could be reproduced experimentally or not.

EXPERIMENTAL PRODUCTION OF SIMILAR GLOBULES IN RATS

A white rat, weighing 220 gm., was injected intramuscularly with four successive doses of bismocymol as follows.

On February 8, 1933, 0.1 cc. (equivalent to 261 mg. pro kg.) was injected, on February 9th 0.2 cc. was injected, on the 10th 0.45 cc., and on the 13th 0.4 cc. The animal showed no symptoms save a loss of weight of 30 gm. It was killed 3 days after the last injection. Bismuth was still present at the site of injection. About the material

was a grayish membrane, outside of which the muscle was edematous and hemorrhagic. The kidneys were dark red, the cortex showing grayish streaking. Other viscera were not abnormal.

Microscopically the kidney is the seat of an intense tubular necrosis. In many of the convoluted tubules the epithelial cells have lost their nuclei, stain intensely with eosin, are partially exfoliated and completely plug the tubules. There are no regenerative changes. Spherical inclusions, identical with those in the human tissue, are present both within the nuclei and in the cytoplasm (Figs. 8 and 9). They give similar microchemical reactions.

A section through the site of injection shows the bismocymol still present in the form of large granular deposits or agglomerations of spherical refractive masses having a greater variability in size than the inclusion bodies within the kidney. Treated with ammonium sulphite solution much of the deposit is immediately blackened. Many of the smaller globules take only a light brownish tint and thus closely resemble those found in the kidney. They are, however, entirely extracellular. With Komaya's reagent the injected material takes a brick red or orange color, rapidly decolorized by alkali but resistant to weak acid treatment. Some of the smaller globules react very feebly to the reagent. This suggests that the bismuth had been split off, leaving a non-reacting residue.

The injected material is surrounded by a broad zone of edematous granulation tissue. Many of the smaller globules and amorphous masses have been taken in by phagocytic cells, chiefly macrophages, but also polymorphonuclears. No globules, however, are found within the nuclei of the phagocytic cells or fibroblasts.

Chemical analysis of the rat kidney showed 0.042 mg. of bismuth in 0.1962 gm. of wet tissue, or 21.4 mg. of bismuth per 100 gm. of dry tissue.

The experiment was repeated on 7 other rats, as shown in Table I.

The inclusions were found in 5 out of 7 rats, that is, in all those surviving more than 4 days. There was no striking correspondence between the intensity of the tubular degeneration and the number of globules. Rats 11 and 15 showed only occasional tubules with necrotic epithelial cells, but intranuclear globules were numerous. The 2 rats with normal kidneys, killed on the 4th day, failed to show the globules.

The particular bismuth preparation used for treatment of the second case was not available for experimentation. Three rats were, however, given intramuscular injections of a similar preparation of sodium potassium bismuth tartrate with butyn suspended in peanut oil in doses equivalent to 153, 182 and 290 mg. per kg. respectively. None of the rats showed evidence of severe injury to the tubular

TABLE I
Summary of Injections

Rat No.	Total amount injected	Bismuth	Number injections	Days	Tubular degeneration	Globules
	cc.	Mg/K				
2	0.2	77.0	1	4	0	0
16	0.7	145.0	2	11	+++	+++
13	0.6	176.0	3	4	0	0
11	0.7	200.0	3	8	+	+++
1	1.15	287.5	4	8	+++	+++
15	0.82	297.0	2	5	0	++
12	1.78	393.0	4	15	+	+

epithelium, and in only 1 were there found characteristic inclusions within the nuclei. Further experiments extending over longer periods are planned. The preparation was either less toxic for the kidneys than the bismocymol or less bismuth was absorbed from the site of injection.

It seemed of interest to determine also whether similar inclusions appeared in the renal epithelial cells in response to injections of a water-soluble bismuth compound. A rat weighing 132 gm. was injected intramuscularly with 0.5 cc. of Loesser's solution of bismuth tartrate, equivalent to a dose of 107 mg. pro kg. The rat died on the 2nd day, showing extensive necrosis of many convoluted tubules but no refractile globules of the type described. Another rat, which received an injection of the same solution (0.178 mg. pro kg.) plus 0.5 cc. of a 10 per cent solution of camphorated oil into the muscles of the opposite leg, also showed severe tubular nephritis but no refractile inclusions.

Dr. G. H. Raizes of the Dermatological Research Laboratories of Philadelphia very kindly put at our disposal a sample of campho-carbonic acid, uncombined with bismuth. This was dissolved in olive oil by the aid of heat and injected intramuscularly into several

rats in amounts up to 150 mg. given in divided doses. No toxic effect upon the kidney was produced and no refractile globules were found within the epithelial cells.

DISCUSSION

Following the intramuscular injection of 0.4 gm. of bismuth in the form of bismuth camphocarbonic acid in olive oil (bismocymol) into a syphilitic patient there occurred a tubular nephritis of moderate intensity, with regenerative change in progress at the time of the patient's death from a metastasizing gastric carcinoma. The unusual and, so far as we are aware, previously unobserved feature, was the presence of refractile spherical bodies within the nuclei and cytoplasm of the renal epithelial cells of the convoluted tubules. A second case, in which the patient had been given 1 gm. of potassium bismuth tartrate suspended in peanut oil, was found to have similar refractile globules in the epithelium of the renal tubules. The histochemical reactions in the two cases were identical.

One can say little that is definite as to the chemical nature of these bodies. Their failure to stain with sudan III or with Nile blue sulphate or osmic acid, and their resistance to lipid solvents shows that they are not simple fats. The negative reaction to the Ciaccio method, and the fact that they are not anisotropic would indicate that they are not composed principally of lecithin-like substance or of cholesterol esters. On the other hand, the positive staining with Spielmeyer's method may suggest a chemical relation to the myelins. That they are not of a protein nature is shown by their prolonged resistance to tryptic digestion and their resistance to strong acid or alkali. The darkening with hydrosulphuric acid and ammonium sulphite suggests that they may contain traces of bismuth, but the more specific histochemical tests with Komaya's reagent and stannous chloride are negative, although the injected material at the site of injection gives clean-cut microchemical tests for bismuth.

Since similar intracellular bodies are not formed at the site of injection and cannot be detected within the glomeruli, one may assume that the material of which they are composed is eliminated from the glomerulus either in solution or in finely dispersed form, reabsorbed by the tubules, and segregated by the nucleus in the form of one or rarely two refractile globules. That the bodies are primarily formed

within the nucleus rather than the cytoplasm is indicated by the appearances in Rat 11 which received in divided doses 0.7 cc. of bismocymol, equivalent to 200 mg. pro kg. Killed on the 8th day after the first injection there was found only a slight tubular injury. Numerous globules were present in the epithelial cells, but almost exclusively within the nuclei. Often their presence was unaccompanied by any other evidence of cell injury.

In searching the literature for similar observations there was found a short paper by Kollert, Strasser and Rosner.³ Following the administration of trepol (potassium sodium tartrobismuthate) there appeared in the urinary sediment small, polygonal, finely granular epithelial cells. The nucleus of these was usually not distinguishable, but a sharply contoured refractile body about the size of the nucleolus was often distinctly seen. No doubly refractile bodies were found with the polarizing microscope.

In the description of the tubular nephrosis produced in rabbits by toxic doses no further mention is made of these refractile bodies. Whether they were of the same nature as those described above or not cannot be decided. We have found no other reference to such structures in the rather extensive literature concerning the effects of bismuth upon the tissues.

SUMMARY

Refractile globules were found within nuclei and cytoplasm of renal epithelial cells in 2 cases following intramuscular injection, in 1 instance of bismocymol (a bismuth derivative of campho-carbonic acid), and in the other of potassium bismuth tartrate with butyn. Similar globules were found in the renal epithelial cells of rats after the injection of appropriate doses of bismocymol. The chemical nature of these globules was not determined; they gave equivocal reactions for bismuth, were insoluble in lipoid solvents and in strong alkalis and acids, resisted tryptic digestion, did not react for iron or calcium but stained as myelin by the Spielmeyer method.

NOTE. We are indebted to Doctors Allen Whipple and Walter Palmer for permission to include the clinical records of these cases.

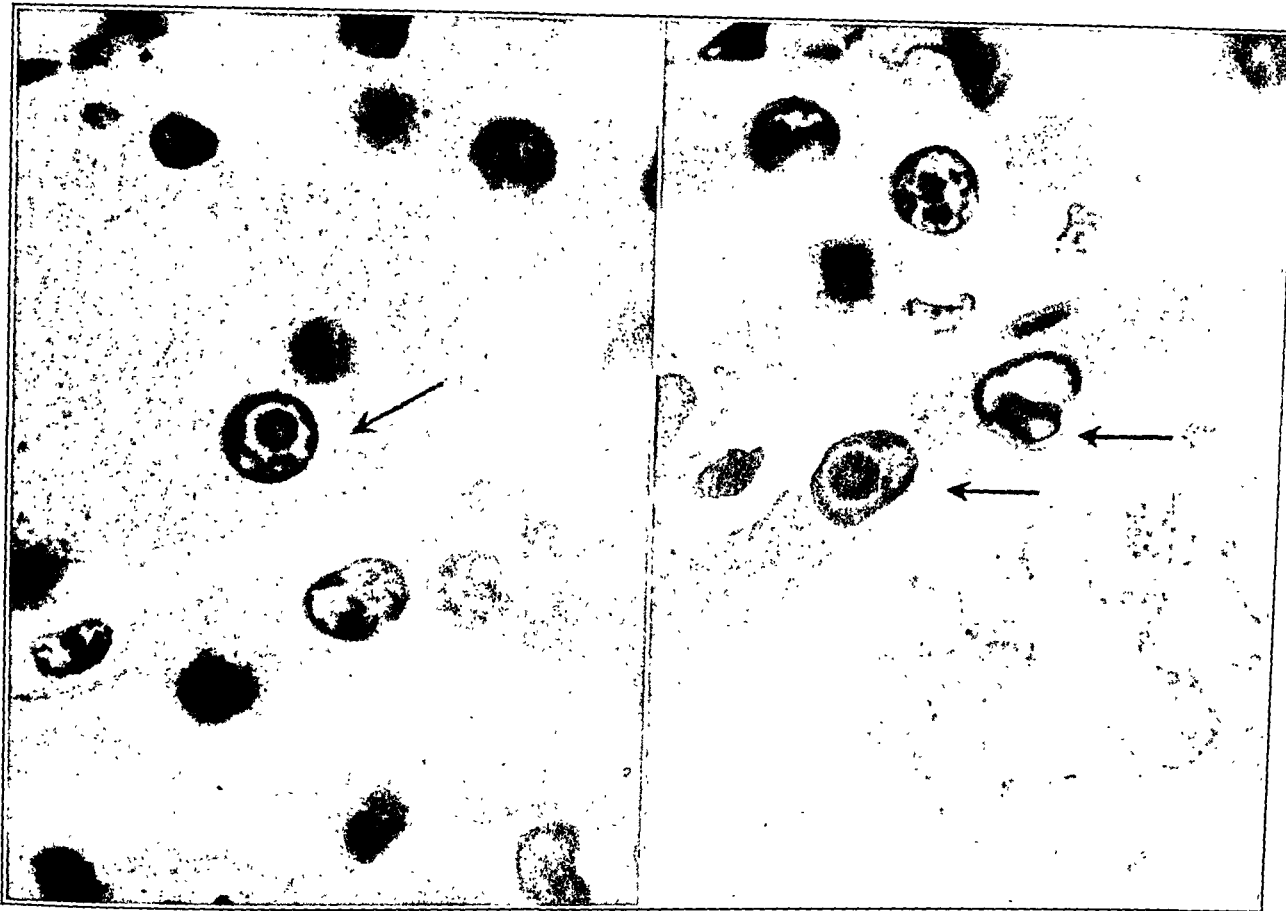
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1. Komaya, G. Über eine histochemische Nachweismethode der Resorption, Verteilung und Ausscheidung des Wismutes in den Organen. *Arch. f. Dermat. u. Syph.*, 1925, 149, 277-291.
 2. Leonard, C. S. Studies in the pharmacology of bismuth salts. I. A method for determination of bismuth. *J. Pharmacol. & Exper. Therap.*, 1926, 28, 81-87.
 3. Kollert, V., Strasser, U., and Rosner, R. Trépol und Niere. *Wien. klin. Wchnschr.*, 1923, 36, 49-50.
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DESCRIPTION OF PLATES

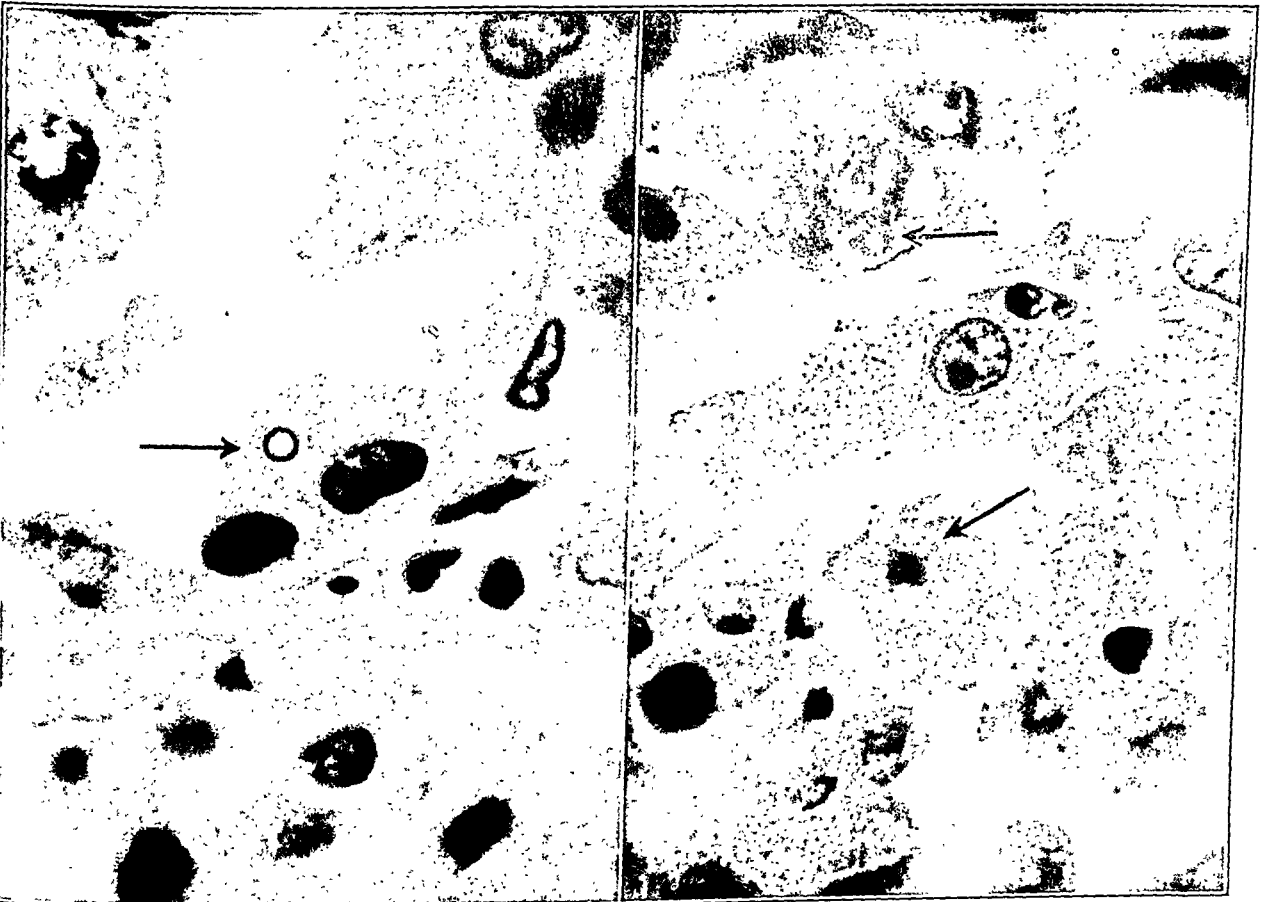
PLATE 138

- FIG. 1. Case 1. Intranuclear body in renal epithelial cells. Hematoxylin-eosin stain. $\times 1680$.
- FIG. 2. Case 1. Two inclusion bodies are seen; one spherical in shape, completely within nucleus; another, pear-shaped, apparently escaping through a gap in the nuclear membrane. $\times 1680$.
- FIG. 3. Case 1. Refractile spherical inclusion in cytoplasm. The nucleus is pyknotic and irregular in shape. $\times 1680$.
- FIG. 4. Case 1. Two cytoplasmic inclusions are shown, one adjacent to a nucleus; another lies in a necrotic cell that has lost its nucleus and is desquamated into the lumen. $\times 1680$.



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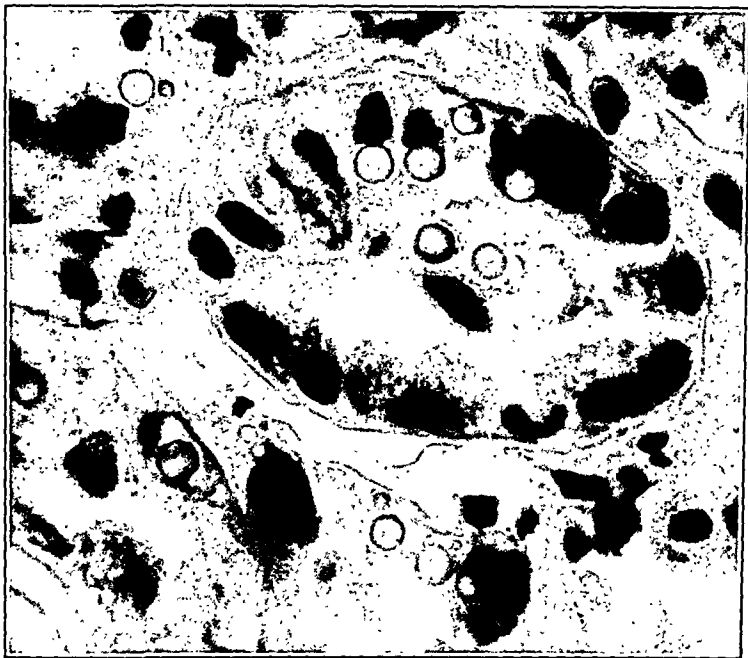


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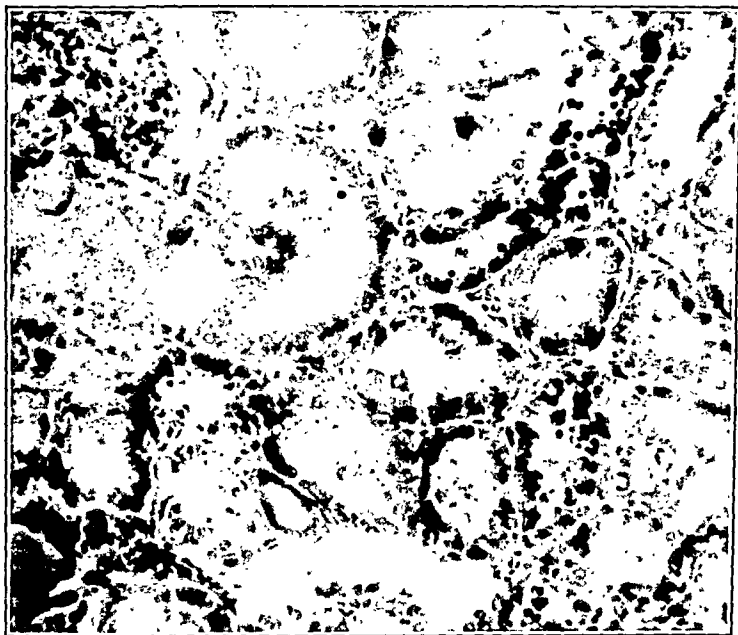
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PLATE 139

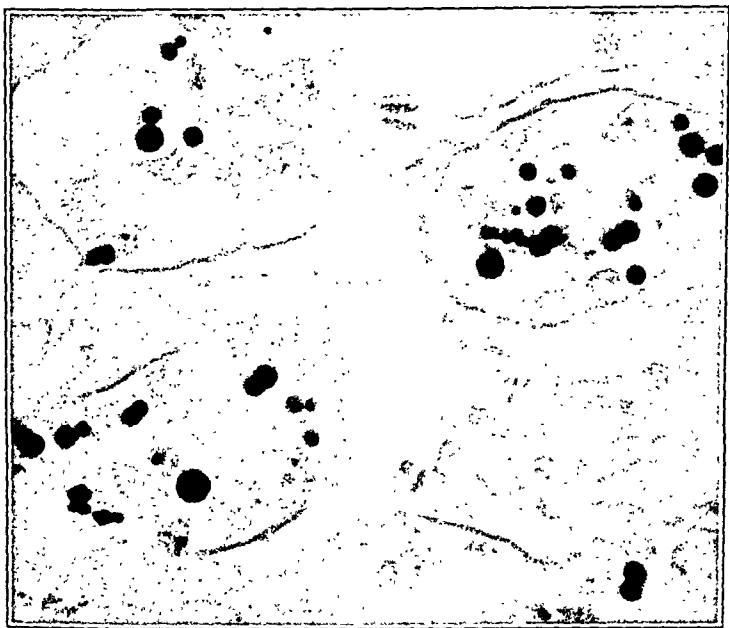
- FIG. 5. Case 2. Renal tubules containing numerous inclusions, chiefly cytoplasmic. Zenker fixation. Hematoxylin-eosin stain. $\times 720$.
- FIG. 6. Case 2. Frozen section of kidney, myelin stain. Spielmeyer's method. The globules are stained black. $\times 100$.
- FIG. 7. Case 2. Formalin-fixed tissue, washed, digested for 12 days with Merck's trypsin. The outlines of the tubules are still recognizable. The globules resist digestion and stain with hematoxylin. $\times 700$.



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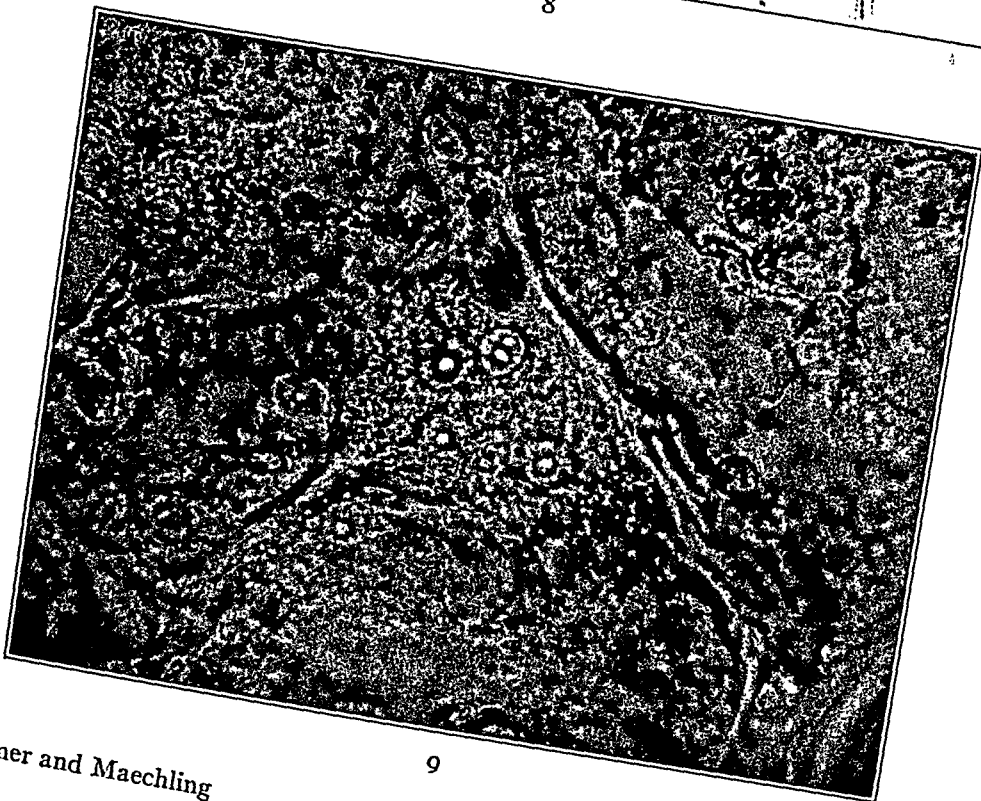
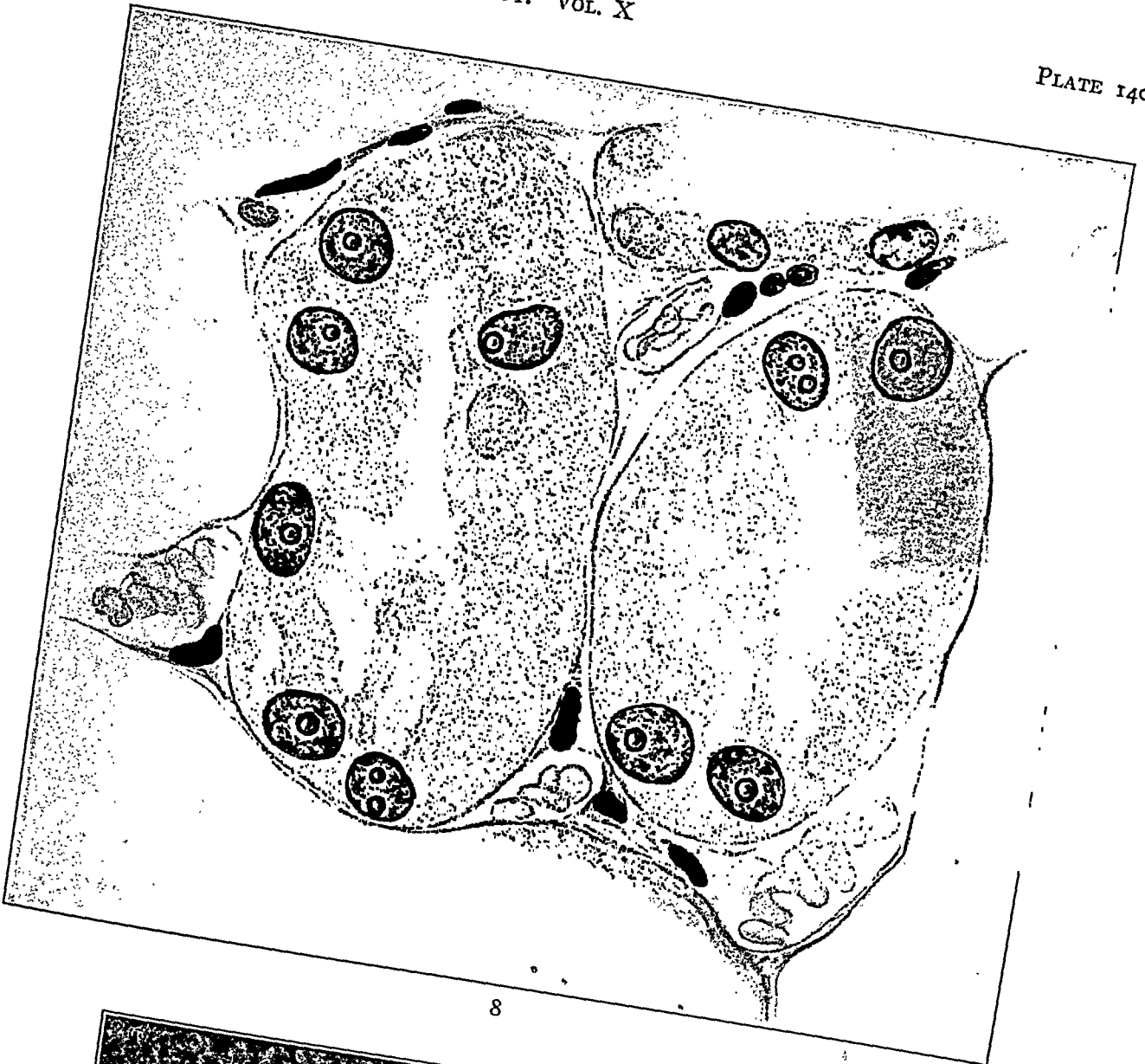


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PLATE 140

FIG. 8. Rat 15. Numerous refractile globular inclusions within nuclei of renal epithelial cells, following injection of bismocymol. Zenker fixation, hematoxylin-eosin stain.

FIG. 9. Rat 15. Kidney with intranuclear inclusions. Unstained frozen section after formalin fixation. $\times 720$.



Pappenheimer and Maechling

Inclusions in Renal Epithelial Cells

GLYCOGEN-STORAGE DISEASE *

THESAURISMOSIS GLYCOGENICA (VON GIERKE)

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Glycogen-storage disease was first described by von Gierke¹ in 1929 when he reported the clinical and autopsy observations on 2 cases. Their most unusual feature was the large size of the liver and kidneys, and the direct cause of enlargement was the accumulation of glycogen in quantities far beyond those commonly observed. Glycogen was found principally in parenchyma cells, a point of difference from most storage disorders where the reticuloendothelial system is the principal depot. In a subsequent report² von Gierke emphasized the storage aspect of the condition and proposed the descriptive name "*Thesaurismosis glycogenica*." His report and the records of 10 proved cases reported since 1929 provide a basis for outlining the main features of this interesting disease. A disorder of infancy and childhood, it seems to be the result of defective metabolism, or at least defective mobilization of carbohydrates. The exact mechanisms concerned are obscure, but we know that glycogenic infiltration is not confined to the organs that are obviously enlarged. The type first described by von Gierke, in which the liver and occasionally the kidneys are hypertrophied and infiltrated with glycogen, has been designated as hepatomegalic or hepatonephromegalic. Bischoff³ and Putschar⁴ first described a cardiomegalic type. We wish to present observations on 4 cases, 3 of them proved and 1 a probable example of cardiomegalic glycogen-storage disease.

CASE REPORTS

CASE 1. *Clinical History:* E. L., a male infant, and the first child of young, healthy parents, weighed 9½ pounds when born on Oct. 20, 1932. Seemingly normal at birth, he took feedings poorly and often was constipated. When 3 months old he had "influenza," after which he coughed for 1 month. He gained weight slowly and weighed 14 pounds at 4½ months. He was brought to the

* Received for publication May 14, 1934.

Bobs Roberts Memorial Hospital on March 15, 1933, because of moderate fever and cough of 4 days duration.

The infant appeared malnourished, dehydrated, listless and extremely weak. His temperature was 37.8° C. The skin was dry and cold and the tissue turgor poor. Cyanosis was observed, breathing was labored, and expansion of the chest was limited on the left side. Râles were heard over both lungs and there were signs of consolidation of the left lung. The heart was enlarged and a soft, blowing systolic murmur was heard at the apex and in the left midaxillary line. A roentgenogram of the chest confirmed the impression that the heart was greatly enlarged and the left lung consolidated. The liver and the tip of the spleen were palpable. The scrotum was large and cystic and the head of the right epididymis was firm. The erythrocyte count was 3,300,000, with 75 per cent hemoglobin. The leukocytes numbered 15,800 with 51 per cent neutrophils, 39 per cent lymphocytes, 8 per cent monocytes and 2 per cent eosinophiles. Examination of the urine was negative except for the presence of a few leukocytes. Wassermann and Kahn tests on the blood of the parents and a Kahn test on that of the infant were negative. The temperature rose to 39.4° C before death, which occurred on March 16, 1933.

The clinical diagnosis was left lobar pneumonia and probable right bronchopneumonia; cardiac hypertrophy with mitral regurgitation, based on an organic (congenital) defect; and bilateral hydrocele.

Autopsy Report

The autopsy was performed 12 hours after death. The body was 64.8 cm. long, and weighed 6.14 kg. The lips and nailbeds were cyanotic. The skin was pale and loose, and edematous only in the scrotum. The chest was symmetrical save for a bulging right costal border below which the liver was felt. The abdomen was distended and the extremities appeared thin. Subcutaneous fat was scanty and the muscles of the trunk were extremely pale. The margin of the liver was 3 cm. below the right costal border. When the sternum was removed the heart was found to be enormously enlarged, with its left border in contact with the lateral chest wall and its right border 1 cm. to the right of the sternum. The left lung was displaced backward and upward. The thymus, a fleshy organ weighing 28 gm. (normal range 6.3 to 19.3 gm.⁵) covered the upper half of the pericardium. The pericardial sac and the left pleural cavity each contained 10 cc. of clear fluid.

The heart weighed 140 gm.* (29 gm.⁶). Measured *in situ* the length from the base of the ventricles to the apex was 10 cm., while

* In this and subsequent notations the "normal" weights (cited in parentheses after those observed) are the mean weights of organs of children in the corresponding age group, as given in the tables of Coppoletta and Wolbach.⁶ Where the age fell between two listed groups the weights for the older group were chosen.

the greatest transverse and anteroposterior diameters were 7.5 and 5 cm. The apex was broad and the left margin and the anterior wall were rounded. The atria and auricles were small, but the walls of the ventricles were firm and thick and their large muscle structures encroached on the cavities. Measured between the muscle columns the right ventricle was 4 to 6 mm. and the left 1 to 1.4 cm. thick. The intact interventricular septum was 1 to 1.2 cm. thick. A probe could be passed through a small channel at the margin of the foramen ovale but the ductus arteriosus was closed. The valve circumferences were: tricuspid 6.2 cm., pulmonic 4 cm., mitral 5 cm., and aortic 3.5 cm. Marginal fenestrations were present in both sets of semilunar cusps. Both the mural endocardium and the epicardium were opaque. The exceptionally pale myocardium was reddish pink mottled with pinkish gray. A wide pale zone beneath the endocardium of the left ventricle had a peculiar glassy appearance. The coronary arteries were large, but like the large arteries and veins they were normally formed.

The lungs were hyperemic and wet and the posterior third of the left lung was atelectatic. Beneath a group of pleural petechiae in the left lower lobe were several small, red, consolidated patches. The liver weighed 260 gm. (188 gm.) and had a smooth capsule. Its tissue was pale yellow-pink, with indistinct lobules, and was soft but not friable or greasy. The two kidneys weighed 60 gm. (50 gm.) and were cyanotic. The spleen, weighing 20 gm. (16 gm.), had firm, red-purple pulp and large malpighian bodies. The lymphoid tissue of the lower ileum and colon was hyperplastic, and the mesenteric lymph nodes were moderately enlarged while the other visceral nodes were small. Together the adrenal glands weighed 3.4 gm. and each had a thin pale cortex and a red-brown medulla. The tunics of both testicles were distended by clear fluid and a cystic hydatid was attached near the head of the right epididymis. The brain was not examined.

Microscopic Examination

Technique: Fourteen hours after death tissues were placed in Zenker's solution and in formalin. Scarlet R was used to demonstrate fat. The complete series of Zenker-fixed tissues was embedded in celloidin and stained with hematoxylin and eosin. Sections of striated muscles were also stained with Mallory's phosphotungstic

acid hematoxylin. Lacking material properly preserved for demonstrating glycogen the tissues fixed in formalin (heart, liver, kidney) were embedded in celloidin and stained with Best's carmine. Subsequently this stain was applied to the Zenker-fixed tissues. Where sections of the same organ were compared glycogen was more abundant in the tissues fixed in formalin. The nature of the granules stained by carmine was checked by the simultaneous staining of control sections. These included tissues known to contain glycogen, glycogen-free tissues, and in some instances saliva-digested sections of the tissues under investigation.

Because of poor fixation and the interval of 1 week between fixing and embedding the tissues, positive observations were more significant than negative ones. Where glycogen was abundant large aggregates of coarse and fine red granules completely filled vacuolated cells, but in other regions their locations suggested diffusion artefacts. We do not know whether or not artefacts are to be blamed for the occasional presence of granules in the interstitial tissues and in the lumens of ducts and blood vessels. Proof that some glycogen had escaped into the formalin preserving fluid was afforded by its milky opalescence. Treated with alcohol this fluid yielded a white precipitate with the physical and chemical properties of glycogen.

Heart: The outstanding features are hypertrophy and vacuolization of the muscle fibers. The largest, up to 50 microns in diameter, lie close to the endocardium, and some of these probably are fibers of the Purkinje type. In the large muscle columns of the left ventricle many fibers measure 30 microns, while in the outer part of the wall most of them are between 20 and 25 microns in diameter. Most of the diameters in the wall of the right ventricle are between 15 and 20 microns, and in the left atrium between 10 and 15 microns. In comparison few fibers measuring more than 12 microns are found in a number of hearts of infants, 5 to 12 months old.

In cross-sections almost all the fibers appear as round or polyhedral spaces surrounded by thin mantles of dots, the cut ends of myofibrils. Where nuclei are transected they are sometimes located in the center of the axial cytoplasm, more often displaced peripherally. They appear normal but some are unusually large. Longitudinal striations are distinct in fibers cut obliquely or lengthwise, but the fibrils often appear teased apart and broken off, especially where they approach large vacuoles. While the appearance of cross-

sections indicates that the axial spaces are continuous, in longitudinal sections they vary in width so that the fibers bulge irregularly. The apparent fragmentation of the fibrils is probably due to changes of direction caused by this bulging. Cross-striations are well defined in some fibers, indistinct where the fibril mantles are thin. Intercalated discs are not seen.

Glycogen granules completely fill many of the fibers, and in others form crescents or rings about clear spaces. Granules are coarser and less uniform in size and distribution than in control sections. They fill every cell in the zone that grossly had appeared glassy. A few fine granules are seen in the interstitial tissue and in the lumens of blood vessels. The muscle fibers do not contain fat or lipoids. The endocardium is moderately thickened and fibrous.

Skeletal Muscles: Comparable changes are found in the skeletal muscles where they are responsible for an unusual type of vacuolar degeneration, which we believe has not been described previously. In some fibers the vacuoles are so large that cross-sections are at first mistaken for fat cells. Similar changes, varying only in degree, are found in the cremasteric, rectus abdominis and intercostal muscles and in fragments of the pelvic muscles. Measurements of fibers of the rectus abdominis are as follows: average diameters for relatively normal fibers 25 microns, for swollen hyalinized fibers 35 microns, range for moderately vacuolated fibers 30 to 45 microns, for extremely vacuolated fibers 50 to 90 microns, with most of them between 50 and 70 microns. "Normal" measurements for comparison are not available. The muscle fibers of the newborn infant are said to measure 6 to 15 microns; the diameters of adult muscles show a wide range, from 17 to 100 microns, with the majority between 40 and 70 microns.

In cross-sections few of the fiber sheaths are filled in orderly fashion by the cut ends of myofibrils. In a few small fibers there are single centrally placed vacuoles, but in the majority the cut ends appear as reticular networks with round or irregular spaces separating fibril bundles. Fibrils are sometimes in contact with the sarcolemma and sometimes separated from it by an empty slit or ring. The peripherally located nuclei of the less vacuolated fibers are normal, but in those with large vacuoles the nuclei often are displaced from the sarcolemma, and often are pyknotic and distorted. Interspersed with fibers of these types are empty or almost empty rings, thought

to be fat cells until longitudinally cut sections failed to demonstrate fat infiltration. The discovery of an intermediate type of fiber identifies these rings as muscle sheaths. In this type the rings contain pyknotic and shrunken nuclei surrounded by halos of granular and fibrillar débris. Some of these tangled fibrils are beaded, almost certainly identifiable as degenerating myofibrils.

In fibers cut lengthwise the cross-striations are distinct when the sheaths are well filled with myofibrils. In many swollen sheaths the fibrils are grouped in thin and irregularly coursing bundles and cross-striations often are hazy or represented by unevenly spaced dots. Obviously the completely empty sheaths cannot be recognized with certainty but the intermediate type is easily identified. In the latter, pyknotic nuclear remnants with their halos of débris are distributed throughout the almost empty, irregularly bulging sheaths. In some, the granular débris is arranged so that it appears to surround vacuoles or unstained granules. In others, a few remnants of myofibrils still show cross-striations.

Coarse and fine glycogen granules are demonstrated in many of these vacuoles and clumps of granules almost fill some of the nearly empty sheaths. In the sections of cremasteric muscle nearly every vacuolated fiber is well filled with glycogen. In other muscles granule-filled fibers are found side by side with seemingly similar fibers in which no glycogen is found.

Liver: All the hepatic cells have pale foamy cytoplasm, and their small vacuoles and cell membranes are outlined by eosin. Most of the vacuoles are smaller than the nuclei, and in a few cells the nucleus is displaced peripherally. The sinusoids are narrow, and the nuclei stain normally. Fine glycogen granules fill many of the cells and in others are concentrated in crescentic masses along one side. Fine granules are present in the epithelium of the bile ducts and in the smooth muscle cells of the blood vessels. A few are seen in the interstitial tissues and in the vascular lumens. Fat is demonstrated in only a few liver cells.

Kidneys: Precipitated protein is present in the glomerular spaces and tubules. The cells of the medullary tubules are unusually pale and swollen and contain considerable glycogen. In smaller quantities glycogen is present in the epithelium of the loops of Henle and of some of the convoluted tubules. As in the liver, vascular smooth muscle cells contain glycogen, and coarse red granules and clumps

lie in the lumens of a few medullary tubules. A few of the large pale epithelial cells contain granules which are stained orange by scarlet R, while red fat droplets are abundant in the epithelium of the cortical tubules.

Lungs: In sections from the left lung we find hyperemia and edema, atelectatic foci and bronchopneumonic patches. A little glycogen is observed in the cells of the bronchial cartilages.

Lymphoid Tissues: In the mesenteric lymph nodes, the thymus, the ileum and the spleen the lymphoid tissues are hyperplastic. In the last two the lymph follicles have centers of large pale cells and fragmented nuclei. Glycogen is found in some of these pale cells and in large cells in the medulla of the thymus.

Smooth Muscles: Axial vacuoles are seen in smooth muscle cells of the urinary bladder, the ductus deferens and many blood vessels, but not in the muscle cells of the ileum. Many of these vacuoles are 10 to 12 microns in diameter and occupy most of the cross-section of the fiber, so that the affected muscle is pale and lacy. No glycogen is found, excepting in the vascular smooth muscle of the liver and kidneys.

Other Organs: Glycogen is not found in the other tissues examined. The thyroid gland has small, colloid-filled acini lined by low cuboidal epithelium. A few small hemorrhages are seen in the medullas of the adrenal glands. The pancreatic islets are not unusual but the acinar cells stain very irregularly because some are well filled with zymogen granules, while others have a pale vacuolated cytoplasm. The nature of these vacuoles, which are often as large as the nuclei, cannot be determined. The genital organs are normal for the age.

CASE 2.* *Clinical History:* G. H., a male twin 8 months old, was brought to the Rockford Hospital on March 27, 1933, because of an acute illness with fever. He had developed normally for the first 2 months, and then had a series of respiratory infections, diagnosed as pneumonia on three occasions. He had been restless, had a poor appetite, and not only had failed to gain, but recently had lost weight. He weighed 12 pounds as compared with the weight of his "control" twin, 19 pounds.

The child was acutely ill and had a fever of 100° to 104° F. The liver was enlarged, and both physical examination and a roentgenogram demonstrated un-

* Our attention was directed to this case by Dr. Joseph Brennemann of Chicago, and we are indebted to the attending physician, Dr. W. L. Crawford, and the pathologist, Dr. H. D. Palmer, both of Rockford, Ill., for the data presented. They also permitted us to examine the heart and prepare sections of the myocardium.

usual cardiac enlargement. Bronchial breathing was heard on the left side posteriorly, but the lung fields visible in the X-ray film were clear. The urine contained no acetone or diacetic acid. Two blood cultures remained sterile. On March 29th the erythrocyte count was 4,400,000 with 79 per cent hemoglobin, and on March 31st 3,650,000 with 78 per cent hemoglobin. The corresponding leukocyte counts were 16,850 and 15,050, with 41 and 51 per cent neutrophils. Cyanosis appeared before death, which occurred on March 31st.

The clinical diagnosis was unexplained cardiac hypertrophy, possibly with endocarditis.

Autopsy Report

The body looked like that of an infant 4 or 5 months old. Edema was absent and the skin was wrinkled because of the scarcity of subcutaneous fat. The abdomen was distended. Each pleural cavity contained 25 cc. of fluid. *In situ* the greatest width of the heart was 9 cm., as compared with the maximum transverse diameter of the chest, 14 cm. The heart was globular and had thick-walled ventricles, small cavities and normal septa and valves. At its thickest part the right ventricle measured 8 mm., the left 3.2 cm. (after fixation 1.7 to 2.2 cm. in the regions between its huge muscle columns). The heart and lungs together weighed 360 gm. and the net weight of the heart was estimated as 260 gm. (37 gm.). The thymus weighed 5 gm. The left lung was compressed and the right contained pneumonic patches. The liver was large, contained much blood, and extended four finger-breadths below the ribs. The kidneys were of normal size.

Microscopic Examination

The findings are very similar to those in Case 1. In the lungs are atelectatic foci, edema, hyperemia and small pneumonic patches. The liver cells are filled with small vacuoles but contain no fat. The tubular epithelium of the kidney is swollen and minutely vacuolated. The general architecture of the myocardium is unchanged, but there is a striking hypertrophy with vacuolization of the muscle fibers without fatty change. Sections were prepared from various parts of the heart, after it had been kept in preserving fluid for 5 months. We find the myocardial fibers differing from those of Case 1 only in that they and their more vesicular nuclei are larger. With the carmine stain their vacuoles are well filled with glycogen. The preserving fluid also contains considerable glycogen.

CASE 3.* *Clinical History:* L. W., a female infant, 4 months old, weighed 7 pounds and seemed normal at birth. For 1 month before admission to the Babies Hospital on Dec. 10, 1922, she had a cough and lost weight. For 1 week her ankles and wrists had been swollen. Fever was absent. She was admitted in a moribund state with physical signs suggesting pulmonary consolidation, and died 9 hours later.

Autopsy Report

The nutritional state was poor, edema was present in the extremities, and there were many small dermal angiomas. The heart, large and globular, weighed 90 gm. (27 gm.). The left ventricle was 1.5 to 2 cm. thick, the right 5 to 9 mm. The papillary muscles were large and the ventricles were not dilated. The myocardium of the right ventricle was firmer than that of the left and was pale pink. The lungs contained regions of congestion and of atelectasis, and were emphysematous anteriorly. The liver and kidneys were congested and were not enlarged. The liver was flabby and seemed somewhat fatty. The spleen was firm and contained conspicuous malpighian bodies. The lymphoid tissues were not hyperplastic. A few petechiae were seen in serous membranes.

Microscopic Examination

Sections of the liver and heart are almost identical with those of the preceding cases. There is little fat in the liver. The muscle fibers of the diaphragm are vacuolated, resembling the moderately vacuolated skeletal muscle of Case 1. There is swelling of the epithelium of some of the renal tubules. The spleen has large malpighian bodies with centers of large pale cells and there are similar cells in the pulp. According to the protocol glycogen was demonstrated in formalin-fixed sections of the heart, but not in those of the liver.

CASE 4.† *Clinical History:* K. B., a female infant, 5½ months old, was admitted to the Harriet Lane Home on May 7, 1919, because of cough, weakness, restlessness and loss of weight during the past 2 months. This baby was weak from birth and had pneumonia at the age of 7 weeks. She was fairly well developed but the nutritional state was poor. The left side of the chest bulged

* This case is included with the consent of Dr. Martha Wollstein of New York City, who sent us microscopic preparations and the protocols. Both this and the following case were found by one of us in a survey of hearts showing unexplained hypertrophy.

† This case, which must be classed as presumptive because the presence of glycogen was not proved, is included by permission of Dr. Edwards H. Park and Dr. Arnold Rich of Johns Hopkins University.

and showed diminished expansion and dullness. No râles were heard and the heart sounds were clear and regular. A roentgenogram confirmed the suspected cardiac enlargement and revealed clear lung fields. The spleen and the liver were palpable. Dyspnea, high fever and increasing prostration preceded death at the age of 6 months.

The clinical diagnosis was idiopathic hypertrophy of the heart.

Autopsy Report

The heart weighed 128 gm. (31 gm.). The wall of the right ventricle was 5 mm. thick, that of the left 1.7 cm. The papillary muscles were large. Valves and septa were normal and the ventricles were roomy but not dilated. There was no evidence of pneumonia. The liver weighed 290 gm. (200 gm.), the spleen 10 gm. (17 gm.) and the two adrenal glands 4 gm.

Microscopic Examination

The hypertrophy and axial vacuolization of the myocardial fibers are of the type seen in the other three hearts. Transverse striations are often indistinct and in some fibers the myofibrils appear finely granular. The muscle cell nuclei are vesicular. The hepatic cells and the cells of the collecting tubules of the kidneys are pale and foamy. The lymphoid bodies of the spleen are small but some have centers of large pale cells. The lungs are edematous but no pneumonic foci are found.

REVIEW OF THE LITERATURE

Proved Cases of Glycogen-Storage Disease

Because the criteria for clinical diagnosis are not well established, only those cases where unusual quantities of glycogen have been demonstrated in hypertrophied organs can be listed as proved cases. Von Gierke's¹ 2 cases were examples of the hepatonephromegalic type of the disorder. Chemical analyses of the tissues of his first patient were reported by Schönheimer.⁷ Unshelm⁸ (Case 2) and Kimmelstiel⁹ described a case which might be designated as hepatomegalic, since the only significantly enlarged organ was the liver. Beumer and Loeschke,¹⁰ Loeschke,¹¹ and Schall¹² added 2 cases of the same type, where the diagnoses were based on hepatic biopsies. Bischoff³ and Putschar⁴ reported the first example of the cardiomegalic type. Pompe¹³ described a similar case, studied chemically

by van Creveld.¹⁴ Pompe also gave brief notes on 3 other cases similar to his own. Antopol, Heilbrunn and Tuchman¹⁵ have recently described 1 case, and the case reported by Sprague, Bland and White¹⁶ should almost certainly be included in this group*. With the 3 cases here reported there are 5 proved examples of the hepatic-renal or hepatic type and 10 of the cardiac type. The essential data are presented in the following summary and table.

Summary: Aside from some of the exceptionally large percentages of glycogen the most unusual feature has been the extent of enlargement of the most affected organs. Livers weighing three to four times and hearts three to seven times the normal weights have been described. These figures are conservative, since the "normal" weights represent mean values for organs of children in the same age groups, while most of these children were subnormal in development and nutritional state. In most instances death was preceded by a severe respiratory infection. There were no significant anatomical abnormalities. Fats and lipoids were present in moderate amounts in some of the glycogen-rich organs, but often were absent. Their presence may have been partly responsible for hepatic enlargement in some cases.¹ Inflammatory lesions were not associated with glycogen-storage and usually there were no proliferative changes. However, two of the affected livers had an increase of fibrous tissue, suggesting an early cirrhosis,^{1, 8, 9} and in our Case 1 the endocardium was thickened. Retrogressive changes accompanied by nuclear degeneration were definite only in the skeletal muscle of the same patient. Possibly the indistinct and granular appearance of the myofibrils in some of the hearts represented early degenerative change. The thymus and lymphoid tissues have been described as hyperplastic and as atrophic. The endocrine organs usually have been considered normal, although in 1 case the adrenal glands were thought to be small¹ and other observers concluded that there were slight abnormalities in the islets of the pancreas. In most of the cases the brain was not studied in detail but in 1 instance it was especially rich in glycogen.^{8, 9}

Aside from the greatly enlarged organs there were differences in the distribution of glycogen. It was found in the heart in only 1 case

* A personal communication from Dr. Tracy B. Mallory, who performed the autopsy, states that carmine-stained granules were demonstrated in nearly every vacuolated fiber of the formalin-fixed myocardium.

TABLE I
Proved Cases of Glycogen-Storage Disease

Case	Age	Sex	Liver		Kidneys		Heart		Glycogen in other tissues
			Weight or size	Glycogen	Weight or size	Glycogen	Weight or size	Glycogen	
von Gierke ¹ Case 1 Schönheimer ⁷	8 yrs.	F	gm. 2000 *(736)	per cent 10.43 †(33.72)	gm. 245 (149)	per cent 6.53 †(36.82)	gm. 80 (110)	per cent ?	A little in skeletal muscle, car- tilage cells, thymus
von Gierke ¹ Case 2	4 yrs. 10 mos.	M	1860 (596)	4.32	Enlarged	Abun- dant	Slightly enlarged	?	In leukocytes
Unshelm ⁸ Case 2 Kimmelstiel ⁹	20 mos.	M	1600 (370)	14.20 †(47.68)	90 (87)	0.81	50 (56)	Abun- dant	Abundant in skeletal muscle, brain
Beumer and Loeschke ¹⁰ Loeschke ¹¹	3 yrs.	M	Greatly enlarged	Abundant (biopsy)
Schall ¹² Case 1	8½ yrs.	F	Greatly enlarged	Abundant (biopsy)	None found in skeletal mu- sle (biopsy)
Bischoff ³ Putschar ⁴	4 mos	F	240 (160)	Abundant	50 (43)	Abun- dant	110 (27)	†Abun- dant
Pompe ¹³ van Greveld ¹¹	7 mos.	F	Not enlarged	9.13	Not enlarged	Abun- dant	100 (34)	7.96	Skeletal muscle 0.30%; spleen 1.46%; adrenals 1.25%; spi- nal cord 0.58%; lung 0.34%

Pompe ¹³ (van Rijssel)	6 mos.	?	?	?	?	?	?	?	Amount not stated
Pompe ¹³ (Deelman Case 1)	4 mos.	?	?	?	?	?	?	?	Abun- dant
Pompe ¹³ (Deelman Case 2)	9 mos.	?	?	?	?	?	?	?	Abun- dant
Antopol, Heilbrunn and Tuchman ¹⁵	4½ mos.	M	Enlarged	§3.25	Enlarged	§ 4.34	?	?	§3.57
Sprague, Bland and White ¹⁶	7 mos.	F	?	?	?	?	?	?	Abun- dant
Humphreys and Kato Case 1	5 mos.	M	260 (188)	Abundant	60 (50)	Abun- dant	?	?	Abun- dant	Abundant in skeletal muscle; a little in cartilage cells, smooth muscle, lymphoid tissue, thymus
Case 2	8 mos.	M	Enlarged	?	Not enlarged	?	?	?	Abun- dant
Case 3	4 mos.	F	Not enlarged	?	Not enlarged	?	?	?	Present	Skeletal muscle?

* Normal weights (in parentheses after observed weights) from the tables of Coppoletta and Wolbach * represent the mean organ-weights of children of corresponding ages.
† Percentage of weight of dried tissue.

‡ Probably about 6 per cent glycogen.
§ Analyses of organs kept in formalin for 1 month.
|| Type of vacuolization suggestive of glycogen infiltration.

of the hepatic group,^{8,9} but we are unable to state whether it was sought in the other 2 fatal cases. In the kidneys of the first child, and in those of infants of the cardiomegalic group, moderate quantities of glycogen were found, mainly in the epithelium of the medullary tubules. In contrast, von Gierke found much glycogen, chiefly in cortical tubules, in the enlarged kidneys of his two patients.¹ Further evidence of variation from case to case is offered by the comments on skeletal muscles. These were reported as containing little,¹ much,^{8,13} and no glycogen,¹² the last the report for an alcohol-fixed biopsy specimen. Part of the variability is probably attributable to technical imperfections. In several cases where glycogen was demonstrated only in the heart it seems likely that the pale foamy cells of the liver and kidneys also contained this substance; at least these cells were similar to hepatic and renal cells in which glycogen was abundant.

The resistance of the glycogen in these tissues to postmortem hydrolysis has attracted much attention. It has been demonstrated in large amounts despite such unfavorable factors as preceding febrile and toxic states and intervals up to 24 hours between death and autopsy. Some of the large percentages recorded in the table represent analyses of tissues kept for several days in the ice-box.^{7,14} In 1 case hepatic glycogen decreased only from 14.2 to 13.67 per cent after 7 days storage.⁸ Schönheimer⁷ found very little glycogenolysis in a liver incubated for 1 week, while the glycogen of a control specimen decreased to one-fifth of the original. Van Creveld¹⁴ observed a decrease of cardiac glycogen from 7.96 to 6.74 per cent after incubating for 3 days at 37° C, and to 1.7 per cent in the same interval, after mixing with normal cardiac muscle. Other observers noticed the same susceptibility to digestion by the enzymes of normal tissues, and, after extraction, by saliva.⁷

Other findings of interest were increased blood glycogen,^{1,14} high blood diastase,¹ and liver amylase.⁸ The possibility of familial occurrence was strongly suggested in 2 cases.^{8,16}

Probable Cases of Glycogen-Storage Disease

Hepatic Type: Recently reported cases which resemble in many respects the proved cases of this type have been described by Snapper and van Creveld,¹⁷ van Creveld,^{18,19,20} Thoenes,²¹ Exchaquet²² (3 cases), Schall¹² (2 cases), Hertz,^{23,24} Biedermann and Hertz²⁵ Zel-

son and Rauh,²⁶ Worster-Drought and Weber,²⁷ Warner,²⁸ Smith and O'Flynn²⁹ (2 cases), and Unshelm (Case 1).⁸ With the exception of the last 3 these children were living when the reports were published. Their ages ranged from 20 months to 12 years. Both sexes were represented almost equally. A number of the older children were improved and seemed to be recovering after periods of observation covering several years. Evidences of hypophyseal dysfunction were thought to be present in 2 cases.^{17, 23} Unshelm's⁸ 2 patients were siblings, as were Smith and O'Flynn's²⁹ 2 patients and Exchaquet's²² 3. Interestingly the last-named were 4 year old twins of different sexes and their 12 year old brother, who seemed to be recovering from a disturbance similar to theirs.

The only autopsy in this group was reported by Smith and O'Flynn.²⁹ An undernourished boy, 6 years old, had a liver weighing 2500 gm. (642 gm.) which had "plant-like" pale cells. They failed to demonstrate glycogen (method?) but found only a little fat in the vacuoles. They quoted the report of the autopsy on the patient's 20 months old sister who had a very large liver "in a state of extreme fatty degeneration." This report did not specify the use of fat stains. That the large fatty liver and the large glycogen-rich liver may be confused is evident from the original diagnosis in von Gierke's Case 2.¹ In 2 other cases^{8, 17} a diagnosis of enlarged fatty liver was made at exploratory operation but was not confirmed by microscopic study.

Cardiac Type: No case of this type has been diagnosed during life. Since almost every heart in the group of proved cases was originally thought to be an example of idiopathic cardiac hypertrophy we might expect to find other cases of glycogenic cardiomegaly masquerading under this diagnosis. We have little doubt that our Case 4 should be listed here, and other hearts with suggestive descriptions are the one reported by Frola³⁰ and possibly that of Steiner and Bogin.³¹ However, a review of the histological descriptions in other case reports and the study of other enlarged, normally formed hearts have convinced us that a group of unexplained or idiopathic cardiac hypertrophies remains.

One other class of abnormal hearts, which may include examples of glycogen-storage disease, is the group of the cardiac rhabdomyomas, particularly the diffuse type. Case 3 of this report was tentatively placed in this group. Certainly Schmincke's³² description

reads like the descriptions of the hearts we have studied. Rehder's³³ case seems to represent a transition between the diffuse and the more common nodular rhabdomyoma. Pompe¹³ has suggested that even the nodular forms may represent localized glycogen-storage disease as their vacuolated fibers are similar and have been shown to contain glycogen. In our cases, however, there were none of the supposedly characteristic "spider cells" (Cesaris-Demel) and none of the appearances suggesting the evolution of myofibrils, so well described by Wolbach.³⁴ The absence of these features and of the extracardiac lesions commonly associated with rhabdomyomas, and the presence of vacuoles in skeletal as well as cardiac muscle, led Dr. Wollstein to doubt whether her specimen (Case 3) should be classified as diffuse rhabdomyoma.

The question of the possible survival to later years of patients with glycogenic cardiomegaly is raised by the reports of Uehlinger³⁵ and Levy and Rousselot.³⁶ Uehlinger attributed to diffuse cardiac rhabdomyomatosis the otherwise unexplained cardiac hypertrophy of a 20 year old laborer who died of tetanus. The heart weighed 340 gm. and its large, vacuolated, fat-free fibers contained much glycogen. It was thought to resemble Rehder's specimen more than Schmincke's. The other patient, an 18 year old schoolboy, died of coronary embolism. The entire myocardium of the 750 gm. heart showed a peculiar vacuolar or "hydropic" degeneration, and few vacuoles contained fat. There were fibrous scars and infarcts in this heart, without evident arteriosclerosis. Although large, vacuolated, glycogen-rich fibers often may be demonstrated at the margins of infarcts, this report emphasized the universal distribution of vacuoles. Moreover, save for scarring, the photomicrographs accompanying the report are almost identical with those of our cases.

Possibility of Other Types: Van Creveld¹⁹ discussed the possible occurrence of types with other major localizations and of local types. He cited Deelmann's observation of glycogen-rich, hypertrophied smooth muscle cells, once in the pylorus of an infant with congenital pyloric stenosis and once in that of a woman 31 years old, who had gastric complaints from infancy. Van Creveld failed to find glycogen in a second case of infantile pyloric stenosis. This association of hypertrophy and glycogen-richness may or may not be significant. We know little of glycogen in smooth muscle, save that it may be found in normal cells.³⁷

Effects of Glycogen-Storage Disease

Cardiac Type: For the most part these infants were normal at birth, but weakness and retardation of development were noticed early and difficulties in feeding were common. Respiratory infections were frequent, often recurrent. Less constantly observed abnormalities were hepatic enlargement, constipation, flabbiness of the muscles, periods of rapid breathing and of cyanosis, tachycardia, cardiac murmurs and edema. The only electrocardiogram showed a sino-auricular tachycardia and normal axis deviation.¹⁶ No metabolic studies have been made, but in contrast to the other group, ketonuria has not been observed.

Hepatic Type: These children, too, seemed normal at birth, but after periods of several days to 1 year abdominal or hepatic enlargement was noticed. Retardation of growth was evident after a few months, with height increase lagging more than weight increase. In some cases the bones were delicate and epiphyseal differentiation was delayed. Usually the muscles seemed fairly well developed but flabby, and the amount of subcutaneous fat was variable. Often walking was delayed. Mental development was unaffected and symptoms referable to the heart and kidneys were negligible. Carbohydrate hunger was a symptom often commented on by the parents. Many of these children had recurrent infections, usually of the respiratory tract.

Metabolic studies were most complete in the 2 proved cases diagnosed by biopsy^{11,12} and in certain of the presumptive cases. Some of the abnormalities of the carbohydrate metabolism are surprising in view of the large stores of glycogen present in the body. The blood sugar in the fasting state has always been low, usually less than 60 mg. per cent. Moreover, even with concentrations as low as 19.5 mg. per cent (Folin) the symptoms commonly associated with spontaneous hypoglycemia were absent. Usually a ketonuria was present in the fasting state, and ketone bodies disappeared slowly after eating. In a few instances ketosis was associated with vomiting. One of the most unusual features has been the failure of adrenalin to induce an appreciable increase of blood sugar or blood lactic acid, and to diminish ketonuria. This refractoriness to adrenalin is in contrast to the hypersusceptibility to insulin, small doses of which lowered the blood sugar and induced the symptoms of hypoglycemia, absent with

the spontaneous hypoglycemias. After ingesting dextrose the blood sugar curves have commonly risen to levels high in relation to the fasting values, but well below those observed in diabetes mellitus. The elevations have been prolonged, sometimes biphasic, and usually maintained for 3 or more hours. Even with relatively large amounts of dextrose glycosuria has not been observed. Other sugars gave similar curves and there has been no evidence of impaired tolerance for galactose and levulose. Tests for other types of impairment of hepatic function have failed to show significant deviations from normal. High normal or increased amounts of blood glycogen have been found, and while the data are not altogether in agreement, there have been no striking abnormalities of the diastatic ferments of the blood. Some observers have reported marked increases in the urinary excretion of diastase.

DISCUSSION

With 15 proved and many probable cases reported since 1929 glycogen-storage disease probably is not a condition of extreme rarity. It is not impossible that milder types, transitory phases and other anatomical varieties of this disorder may occur. The elucidation of its mechanisms and characteristics requires the collaboration of the clinician, the chemist, the physiologist and the pathologist.

An immediate problem for the pathologist, suggested by the inadequacies of many of the methods used, is the proper handling of the tissues secured by biopsy or at autopsy. Perhaps it is superfluous to mention the desirability of chemical analyses, although we failed to appreciate it at the time of our autopsy. Satisfactory methods of preserving tissues and the use of proper controls of staining technique are essential to prevent erroneous conclusions, especially as to the absence of glycogen. While other fluids may be superior we have found that one of the best simple solutions for preserving glycogen in tissues is the one recommended by Bartelmez and Bensley³⁸ and by Burghgraeve.³⁹ This is a mixture of absolute alcohol and formalin, 9 parts to 1. Best's carmine is the most dependable stain and may be applied to sections embedded either in paraffin or in celloidin. Some artefacts seem to be inevitable but errors of interpretation may be avoided by using control sections known to contain glycogen, and sections treated with saliva for 30 minutes before staining.³⁸

The lack of "normal" standards makes it difficult to interpret the significance of the moderate amounts of glycogen found in the non-hypertrophied organs. Of the many reports in the literature few attempt to correlate glycogen content with such factors as age, cause of death, interval between death and autopsy, and so on. Systematic studies prove that these factors are important.^{40, 41, 42} On the other hand, the amounts of glycogen found in some of the enlarged organs are surprisingly large. Compared with Fränkel's⁴³ figures for normal skeletal muscle 0.5 to 2 per cent (the latter for the glycogen-rich muscle of the horse), the 9.39 per cent of glycogen found in the skeletal muscle of Pompe's patient by van Creveld¹⁴ is amazingly high. The cardiac muscle of the same child contained 7.96 per cent of glycogen. Cardiac muscle is said to contain as much glycogen as skeletal muscle, possibly more, but loses it more quickly through postmortem glycogenolysis. The highest value we have found is 1.2 per cent for the heart of a dog after carbohydrate feeding.⁴³ The normal human kidney is supposed to contain very little glycogen. In diabetes mellitus Popper and Wozasek⁴⁴ found 1.64 per cent the maximum value for renal glycogen in their series (26 cases), while Schönheimer⁷ observed nearly four times that amount, 6.53 per cent, in the kidneys of von Gierke's first patient. The figures 9.13 per cent,¹⁴ 10.43 per cent,⁷ and 14.2 per cent,⁸ certainly represent large quantities of hepatic glycogen, although similarly high values are said to occur in other conditions. However, Popper and Wozasek's⁴⁴ analyses of 177 human livers yielded maximum values of 6.17 per cent after sudden death, and 8.50 per cent in diabetes mellitus. In analyses of the livers of 44 infants and children "made at the time of death" Burghard⁴⁵ reported a maximum of 5.2 per cent (diphtheria), and found only 5 with glycogen in excess of 2 per cent. In feeding experiments in animals, however, values above 20 per cent have been attained.^{43, 46}

The identity of the cardiac and hepatic types of glycogen-storage disease is not proved, although it seems probable that the basic defect is a common one. The absence of frank cardiac enlargement does not exclude moderate involvement of the heart in cases of the hepatic type, such as was seen in the youngest child in the group.^{8, 9} Many, if not all, of the children in the cardiac group had a similarly moderate involvement of the liver and kidneys. The strikingly earlier mortality in the second group may mean merely that the child

with cardiomegaly is more seriously handicapped and withstands infections poorly. We do not know whether or not with survival and seeming recovery there are residual metabolic or anatomical abnormalities. All that we know of the metabolic functions of the infants with cardiac disease is the fact that the ketonuria so characteristic of the hepatic type may be absent. On the other hand, even some of the "cardinal" symptoms of the hepatic type may be secondary, a statement suggested by the experiments of Junkersdorf.⁴⁶ By forced feeding with carbohydrate or with fat he was able to produce enlargement of the liver and hypoglycemia in young dogs. He regarded the hypoglycemia as hepatogenic, caused by overloading the liver cells with glycogen or fat, resulting in "mechanical" interference with their blood sugar-regulating functions. Obviously the separation of the basic from possible secondary metabolic defects would be facilitated by metabolic studies in non-hepatic types of the disease, if they could be recognized during life. Our Case 1 suggests a possible usefulness for biopsies of skeletal muscles in identifying such cases.

The removal of even a limited number of conditions from the unsatisfactory category, "idiopathic," encourages the investigation of other unexplained disorders of childhood. In view of the observations already made we might well examine critically the rare cardiac rhabdomyomas,¹³ hypertrophic pyloric stenosis,¹⁹ and the non-neurogenic diseases of skeletal muscle, especially the progressive muscular dystrophies. Obviously we must avoid the error of attributing too much significance to the presence of small amounts of glycogen, even in enlarged organs. It is equally obvious that conditions characterized by vacuolization should not be designated as "fatty" or "hydropic" without an attempt to identify the contents of the vacuoles. The search for glycogen in the tissues of the child with glycogen-storage disease is facilitated by its presence in such large quantities and by its resistance to spontaneous glycogenolysis. Furthermore, experience has shown that the search is not necessarily futile, even with tissues that have been improperly fixed or preserved for some time. Our own experience has confirmed the statement of Bartelmez and Bensley³⁸ that glycogen may be surprisingly well preserved in tissues fixed in Zenker's solution and embedded in celloidin.

It is essential to recognize that many symptoms and even combinations of some of the more unusual symptoms of glycogen-storage

disease may be shared by quite different diseases. This is true of certain types of hepatic cirrhosis, the peculiar syndrome described by Parnas and Wagner⁴⁷ and the famous case of Wilder and his co-workers.⁴⁸ Parnas and Wagner's patient had many symptoms resembling those of glycogen-storage disease, but eventually developed typical diabetes mellitus. Wilder's adult patient had persistent hypoglycemia and was refractory to adrenalin with respect to its blood sugar-elevating function. The point of special interest for us is that this refractoriness did not imply glycogen-impoverishment. At autopsy an insulin-secreting carcinoma accounted for the hypoglycemia, and the liver contained 8.25 per cent of glycogen. These examples merely serve to emphasize the fact that, at least for the present, the certain identification of glycogen-storage disease rests on the examination of tissues obtained at autopsy or by biopsy.

The most common assumption to account for the peculiarities of glycogen-storage disease has been that it represents a prolongation of fetal or infantile behavior with respect to the metabolism of carbohydrates. It is quite generally recognized that glycogen is abundant in the tissues of the fetus, even in tissues that contain little glycogen in the mature organism. The glycogenolytic capacity of fetal tissues is said to be slight. Furthermore, clinical evidence indicates that the carbohydrate-mobilizing mechanisms are poorly developed in the infant. In the proved cases of glycogen-storage disease morphological changes, which would implicate the endocrine organs and the nervous structures concerned in carbohydrate metabolism, have been lacking. Most observers have concluded that the defect probably lies in the affected tissues. Among the explanations offered are deficiency of ferments, binding of enzyme or protection of substrate, the presence of a heterotypical glycogen, and inhibition of hydrolysis by unfavorable reaction or by the accumulating products of hydrolysis. To understand the mechanisms we certainly need a more exact knowledge of normal carbohydrate metabolism, especially with respect to the mobilization of glycogen, than we have today.⁴⁹ The fact that glycogen-storage disease occurs as an infantile disorder, probably congenital, and possibly familial, invites comparison with the interesting class of metabolic diseases characterized by Garrod⁵⁰ as "inborn errors of metabolism." Interestingly most of these congenital or familial metabolic disorders are attributed to abnormal enzymatic functions.

SUMMARY AND CONCLUSIONS

Glycogen-storage disease (*Thesaurismosis glycogenica*, von Gierke) is a disorder of infancy and childhood, possibly familial, and affecting both sexes. It is characterized anatomically by organ enlargement and the storage of glycogen chiefly, but not exclusively, in the enlarged organs. Two main types have been delimited, glycogenic hepatomegaly or hepatonephromegaly, and glycogenic cardiomegaly. The affected organs may attain great size and may contain glycogen in unprecedented amounts. One peculiar feature is delayed postmortem glycogenolysis and the search for glycogen has been successful in spite of adverse circumstances and imperfect techniques. In only a few instances has glycogen-storage been associated with degenerative or proliferative changes. At least in the hepatomegalic type clinical studies have seemed to point to an impairment of the mechanisms concerned in the mobilization of glycogen and the regulation of the blood sugar. Less is known of the cardiomegalic type.

We present the clinical and autopsy studies of 3 proved cases and 1 probable case of cardiomegalic glycogen-storage disease. The weights of the hearts were 3.3 to 7 times normal, and the myocardial fibers were enlarged, vacuolated, and (in 3 cases) filled with glycogen. It is probable, though proved only in 1 case, that the liver and kidneys were affected similarly but to a lesser degree. In 2 cases the skeletal muscles showed an unusual type of vacuolar degeneration, and in 1 of these the vacuoles contained much glycogen. This report brings the total number of proved cases to 15.

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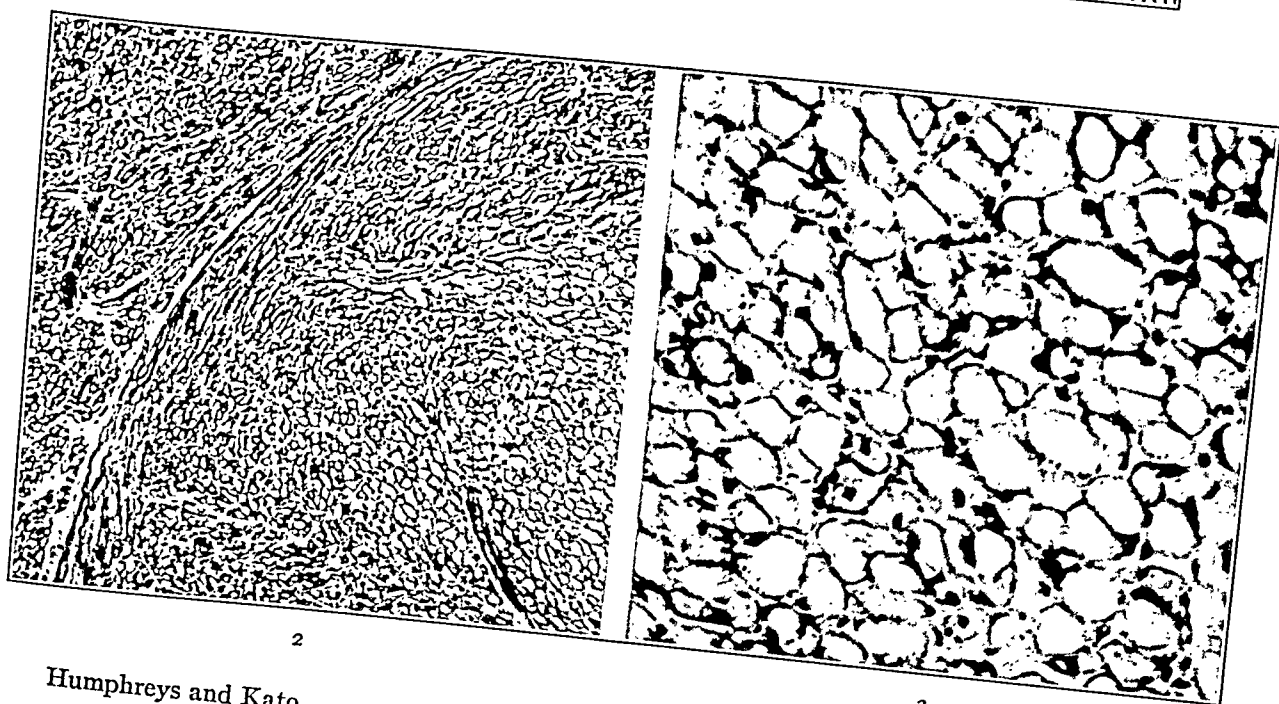
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DESCRIPTION OF PLATE

PLATE 141

- FIG. 1. Case 2. Glycogenic cardiomegaly. This heart weighed 260 gm., about seven times normal for the age, 8 months. The increase in size was due to general enlargement of the organ, without dilatation. Photograph 10 per cent larger than actual size of the fixed heart.
- FIG. 2. Case 1. Glycogenic cardiomegaly. Photomicrograph demonstrates the lacy appearance of the myocardium resulting from the almost universal vacuolization of the muscle fibers. Mallory's phosphotungstic acid hematoxylin stain. $\times 75$.
- FIG. 3. Case 1. Photograph of the large myocardial fibers cut obliquely or transversely. Mantles of myofibrils surround seemingly empty axial spaces. In sections stained with Best's carmine these vacuoles were more or less completely filled with glycogen granules. Mallory's phosphotungstic acid hematoxylin stain. $\times 310$.



2

3

Humphreys and Kato

Glycogen-storage Disease

PLATE 142

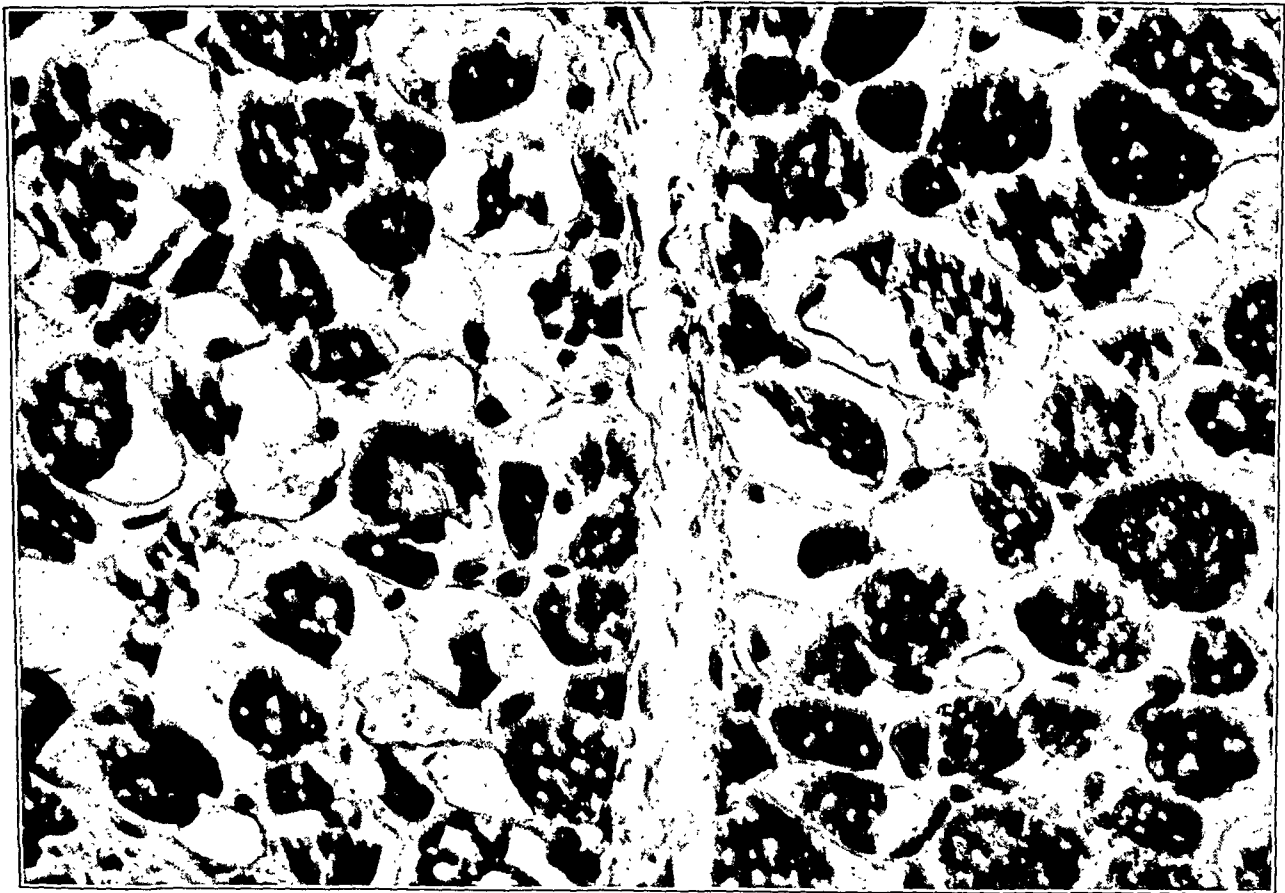
- FIG. 4. Case 1. Skeletal muscle. Photomicrograph of a single swollen muscle fiber cut lengthwise. The bulging sarcolemma contains scattered degenerating nuclei and granular and fibrillar debris. In carmine-stained sections some sheaths of this type were filled with coarse glycogen granules. Mallory's phosphotungstic acid hematoxylin stain. $\times 550$.
- FIG. 5. Case 1. Skeletal muscle. Photomicrograph shows large fibers of the type seen in Fig. 4, alternating with compact, deeply-stained normal fibers and with others whose sparser fibrils appear as if teased apart. Mallory's phosphotungstic acid hematoxylin stain. $\times 550$.
- FIG. 6. Case 1. Skeletal muscle. Photomicrograph of muscle fibers cut transversely to show various degrees of vacuolization and degeneration. The nearly empty sheaths are cross-sections of fibers of the type seen in Fig. 4. The retraction of some of the sarcolemma sheaths may have been caused by shrinkage. Mallory's phosphotungstic acid hematoxylin stain. $\times 550$.



4



5



6

ALTERATIONS IN MINERAL CONSTITUENTS OF ANTERIOR HORN CELLS IN EXPERIMENTAL POLIOMYELITIS *

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The observations herein reported form part of a comprehensive study of the reactions of cells to viruses. The technique is that of microincineration already utilized in this laboratory for investigation of the alterations provoked by the salivary gland virus of guinea pigs (Scott ¹) and the viruses of rabies (Covell and Danks ²), fowl-pox (Danks ³), yellow fever (Cowdry ⁴) and herpes (L. E. and E. J. Rector ⁵). The method, as introduced by Policard ⁶ and modified by Scott,⁷ makes possible the exact topographical localization of the mineral constituents within a single cell, after the removal of its organic constituents by a process of burning. It is easily possible to control the observations by comparing the ashed section with the next following stained section of the same cell. When one considers the important rôle played by inorganic salts in cellular permeability and the colloidal organization of the cytoplasm the importance of accurate data on changes in their distribution is evident.

MATERIAL AND METHODS

Material was obtained from the spinal cords of 43 monkeys experimentally infected with poliomyelitis by intracerebral injection of a virulent cord emulsion. The animals were sacrificed at various periods of nerve involvement ranging from slight fibrillary tremors to complete prostration. Tissue from the spinal cords of 10 normal monkeys was used as control. Pieces 3 to 8 mm. thick were removed from the cervical (fourth to sixth) and lumbar (third to fifth) enlargements and immediately fixed in 10 per cent formalin in absolute

* Aided by (1) the Milbank Fund for the study of Infantile Paralysis, (2) a grant from the Rockefeller Foundation for research in virus diseases, and (3) an appropriation from a grant made to Washington University by the Rockefeller Foundation for research in science.

Received for publication June 3, 1934.

alcohol, Zenker's fluid, and Zenker's fluid without acetic plus 10 per cent formalin. In some cases Zenker's fluid was applied by perfusion through the blood vessels.

The absolute alcohol-formalin fixative was recommended by Scott ⁸ to prevent solution or displacement of inorganic salts in cells prepared for microincineration, while at the same time preserving the cytological structure. Full details of the technique of microincineration are given by Scott.⁹

All sections were cut at 4 microns, since it was found that in thicker specimens the nerve cells showed a marked tendency to shrinkage during incineration. Even at this thickness the outlines of the smaller cells may be contracted in the ashed preparation, but the larger cells reproduce almost exactly the size and shape of the control stained section, or show only such variation as is seen between the outlines of a nerve cell in two consecutive sections. The phenomenon of shrinkage may give rise to a heavy ash about the cell membrane, which may lead to the erroneous impression of a peripheral concentration of mineral matter. In the perfectly prepared specimen such an appearance has not been noted. It was found that slides incinerated on days when the humidity was unusually low presented a clearer picture than those prepared when the air contained more moisture. Even during the short time required to cool a slide the ash particles may sometimes absorb enough moisture to cause a yellowish to dark brown coloration which may be confused with the amber or reddish tint so characteristic of iron-containing tissues. It may also resemble the appearance of sections in which carbon remains after incomplete incineration. The color alteration due to moisture, however, disappears immediately after heating over a Bunsen flame and may be attributable to a change in the refractive properties of the ashed particles brought about by the deposition of a thin film of water on their surface.

After the other fixations the sections were cut 5 microns thick and stained with hematoxylin and eosin, Giemsa's stain, erythrosin-azur or phloxine-methylene blue. The purpose of this was to have at hand an abundance of material prepared by ordinary routine methods to illustrate the well known reactions of the cells to the virus for comparison with the results secured by the new incineration technique. But for making the detailed topographical comparison serial sections of the formalin-alcohol preserved tissues were cut, alternate ones

being mounted with double filtered absolute alcohol (for incineration) and with egg albumin (for staining). Observations were limited to those involving identification of the same cell in consecutive sections after incineration and staining. This was done by noting its position in relation to the outline of the anterior horn, its proximity to such landmarks as blood vessels, and the similarity of cell outlines in the two sections.

For observation of the specimens two Zeiss binocular monobjective microscopes were set up side by side, one equipped with the usual Abbé condenser for examining stained slides, the other with a Zeiss cardioid condenser for dark-field study of the incinerated preparations. The lens systems on the two microscopes were identical. This permitted accurate judgment of shrinkage, distortion and other changes. As a light source a large Spencer lamp (No. 394) fitted with a Projection Mazda, 500 W, 115 V, General Electric bulb and without a daylight filter was used. When one becomes accustomed to the yellowish light provided by this arrangement it is found that color discrimination is in no way interfered with. To guard against the possibility of confusion from structural color (Mason¹⁰) the dark-field observations were checked with incident light from a lamp adjusted in such manner that the beam fell directly on the specimen. This arrangement, of course, could not be employed when an ordinary immersion lens was used. It was found that some of the bluish color in certain structures was not so marked with direct lighting, giving some support to the theory that this might be due to a refractive phenomenon. It cannot be said, however, how much of the color loss is attributable to the distinctly inferior lighting which is obtained with incident illumination. If, as Mason believes to be true for similar materials, the bluish color of this type of preparation is due to a dispersion phenomenon it is still significant since it indicates a definite localization of cellular substance which differs either physically or chemically from that in adjacent areas.

OBSERVATIONS

Attention was confined to the large somatic motor cells of the spinal cord in an effort to determine the sequence of changes in neural constituents and to characterize each alteration as accurately as possible.

Normally these cells contain rather less inorganic material than the Purkinje and spinal ganglion cells described by Scott.⁸ The cytoplasmic ash is made up of an extremely finely divided and evenly distributed dust-like residue usually of a more or less bluish hue, as already reported (Patton¹¹). In this background are found small masses of heavier, flat white material, which in distribution and shape correspond to the Nissl bodies, as seen in the immediately following or preceding control-stained section of the same cell. Close study shows that these masses have a faint but distinct yellow color — a property that may indicate the presence of iron. The nuclear membrane gives a denser and more pronounced ash than the cell membrane. The nucleoplasm does not leave a uniform fine residue like the cytoplasm. Instead its background has a vacant appearance. Set here and there are a few rather definitely outlined clumps of yellowish residue. These probably remain from the particles of iron-containing material in the nuclei featured by Nicholson.¹²

Three stages, which merge the one into the other, may be recognized in the alterations resulting from virus action. These are represented by the three pairs of figures on the plate. The figure on the right of each pair represents the mineral skeleton of a single cell cut in section and viewed in the dark-field, while the one on the left is the immediately adjacent section of the same cell, not incinerated but colored in the usual way with hematoxylin and eosin.

1. *Stage of Edema (Figs. 1 and 2)*: The first change is what Nissl called "acute swelling." When examined in stained preparations the outlines of the cells are seen to be rather rounded and swollen, but the cellular membranes remain intact. The Nissl bodies are lost by "chromatolysis," first near the nuclei but later throughout the cytoplasm. The cytoplasm becomes very pale and looks uneven. The nuclear changes are noticeable soon after the loss of Nissl bodies and represent the first signs of the severe cell injury, which ultimately ends in death. The nucleus, like the cell body, first becomes swollen but later tends to shrink, with a wrinkling of the nuclear membrane. It may be displaced and occupy an eccentric position. The tendency is toward a peripheral migration of the basophilic chromatin, which remains plastered on the membrane; but some of it may still be seen as small, deeply staining, beady granules or as stellate, irregular masses in a net attached like a spider's web to the nuclear mem-

brane. It is in cells during this stage that the "nuclear inclusions" of Covell¹³ and Hurst¹⁴ first make their appearance. A large one is represented above and to the right of the spherical nucleus and a smaller one above and to the left in Figure 1. They are sharply outlined, spherical globules ranging in size from minute specks to bodies of 3 to 4 microns in diameter and are amphophilic in staining property, but may be made to appear acidophilic by varying the degree of differentiation. Sometimes the inclusions seem to have a darker center with a lighter periphery — probably a refraction phenomenon, because the ring disappears on slightly altering the focus of the microscope. On no occasion has a halo been observed, such as is described by Nicolau and Galloway¹⁵ for the nuclear inclusions in Borna disease. These bodies may be numerous within a single nucleus. As many as 8 or 10 have been counted in one cell. In general it may be said that they tend to be smallest when they are most numerous and *vice versa*. Eventually, in every case, the nucleus becomes completely filled with the granular amphophilic material, in the midst of which an unaltered nucleolus may be distinguished by its basophilic staining properties.

Microincineration shows that there is a gradual and parallel diminution in the amount of mineral matter (Fig. 2). The cytoplasm is represented by a thin, finely divided, evenly distributed ash similar to that left by the non-stainable cytoplasm of the normal cell, but the residue of the Nissl bodies does not appear. The nuclear membrane is rendered more prominent by the peripheral migration of basophilic chromatin and appears as a heavy white line studded with clumps of ash. This observation lends additional weight to Scott's¹⁶ statement that most of the mineral matter of the nucleus is located in the basophilic chromatin. The inclusion bodies are too small to extend through two sections but at this stage in development they yielded a small amount of ash. A few of the yellowish ash masses are identifiable and the large, conspicuous nucleolus yields a dense, flat white residue.

2. *Stage of Increase in Mineral Matter (Figs. 3 and 4)*: Up to this point the nucleus and cytoplasm have shown fairly simultaneous changes, but here the parallelism ceases. When both continue they pursue a similar sequence of alterations in every case but either may outstrip the other in the rate at which they occur. Thus we may find a nucleus in the last stages of degeneration while the cytoplasm

shows only the earliest alteration, a condition that causes considerable confusion, but no inconsistency of the picture.

In stained sections the pallid cytoplasm begins to be marked here and there with clouded areas, the alteration spreads, and soon the entire cell body takes on a deeper color with basic dyes — darker even than the nucleus. The outlines of the cell are sharp and well defined, and although no marked shrinkage may be noted, tend to approach those of the normal neurone. As the hyperchromia develops the cytoplasmic contents, colored in this way, become a mass of deep blue-stained granules, translucent, thickset, filling the entire cell and all its processes. Vacuoles may appear. The granules do not remain unaltered long; they tend to coalesce, resolve or concentrate themselves into small, dense, dark beady masses which are seen against a spongy reticulum (Fig. 3). When viewed with high magnification and strong illumination the larger ones assume the appearance of ringlets, which appearance fades with a slight change in focus. Such rings in other cells were pointed out by Nissl as being the chief characteristic of what he called "*schwere Zellveränderung*," although Spielmeyer observes that he has seen them occasionally in man and less frequently in animals. The possibility that these granules might be pigment was considered. Their size, shape and distribution, as well as their constant presence in alcohol-fixed tissues, tends to rule out pigments of the fatty type. Sections stained with methylene blue alone, in which the granules took the stain intensely in every instance, were studied. As a final check serial stained and unstained mounted sections were examined. Cells that showed the granules in the colored section showed no trace of yellowish coloration in the unstained preparation.

The nucleus, last described as being filled with minute spherules of varying size, meanwhile undergoes some alterations. Without variation in amphophilic staining qualities the spherules coalesce and the nucleus becomes a contracted, partly homogeneous mass. Within it may be seen one or more round bodies of somewhat darker material, one of which may represent the nucleolus, others remains of the nuclear material which have not melted into the mass. Compare the nuclei in Figures 1 and 3.

It is in cells at this stage of degeneration that neuronophagia is first observed. The entire cytoplasm and its formed constituents seem to be engulfed by the phagocytic invaders. But some de-

generating cells escape phagocytosis, for some of them are observed even in the last stages of degeneration without even so much as a marked satellitosis.

Microincineration of such cells reveals a striking change (Fig. 4). The cytoplasmic ash, which formerly had been less in amount than that seen in the normal nerve cell, is increased with the onset of the cytoplasmic clouding. It continues to increase with the granular change, reaching a maximum at the stage in which the deeply stained, shotty, basophilic granules are present. When seen at low magnification attention is immediately attracted by these brilliantly refractile clumps of ash within the anterior horn. Close inspection gives the impression that this dense cytoplasmic ash is piled on the slide. The cell outlines are distinct; there is little shrinkage. The ash shows from the first an increased tendency to arrange itself in clumps, and is composed of coarsely granular, white, glistening, glassy particles. The cell processes share in the increase of inorganic material and are represented as brilliant streaks in the gray matter.

The nuclear picture may be obscured by the overlapping of a rim of cytoplasm or by the scattered bits of ash representing the basophilic chromatin. Some cases may be found, however, in which the cytoplasmic mineral is excluded. Such sections indicate that the amphophilic homogeneous material contains a small amount of inorganic matter in the form of a finely divided, film-like ash, flat white or bluish, and evenly distributed. On some the chalky residue of the nucleolus is seen, not strikingly different from that found in the normal neurone. This is the last stage in which the nucleolus can be identified, in either stained or incinerated sections. What its subsequent fate may be, whether it is absorbed, destroyed by rhexis or extruded, could not be determined.

The neuronophagic foci leave an ash dependent on the amount of cytoplasmic mineral matter of the cell attacked. If a granular hypermineralized cell is being destroyed the incinerated focus presents the brilliant ash characteristic of such cells. Apparently the process of neuronophagia is carried on without change in inorganic constituents. The scavenger cell can incorporate and, as it were, digest the entire abnormal mineral content of the dying neurone.

3. *Stage of Necrosis (Figs. 5 and 6)*: Comparison of Figures 5 and 6 with 3 and 4 will demonstrate how marked are the changes

that occur slowly and by imperceptible gradations in the living animal.

Study of stained sections indicates that as the cells become acidophilic the cytoplasmic granules may be phagocytosed, extruded, or disappear by some not easily explainable process, perhaps by lysis. Similar granules may sometimes be seen in polymorphonuclears near the site of the lesion or extracellularly in the gray matter, especially near necrotic cells. The dead, acidophilic cytoplasmic débris left behind usually contains numerous large vacuoles, which are not illustrated in Figure 5.

The amphophilic and almost homogeneous nucleus becomes more and more acidophilic. This alteration in reaction may occur in cells still containing granules, but generally appears approximately coincident with their disappearance. The final nuclear picture, then, is that of a rounded, acidophilic, clear homogeneous lump separated from the cytoplasmic remains by an empty shrinkage space.

Cells of this type are usually removed by neuronophagia. Others may be seen in which the cytoplasm melts away by a lytic process. In these the acidophilic cytoplasm becomes progressively more vacuolated until it is represented by a delicate web of liquifying material. In such cells the nucleus persists to the end and sometimes may be seen in a pericellular space in which no trace of cytoplasm remains.

In incinerated preparations the cells in this final stage are easily recognized. Compare Figure 6 with Figure 4 of the preceding mineral-rich condition. When for some unknown reason the cells are not phagocytosed like their neighbors the excess of mineral matter is reduced in some other way. They retain, however, in death more than they possess in the first stage of the reaction (Fig. 2). The ash is distributed fairly evenly in the cytoplasmic area but is lacking in the spaces occupied by the vacuoles mentioned. The original location of some of these vacuoles is marked by the absence of ash. The hyaline necrotic nucleus leaves little or no ash.

Another type of cell change is frequently found in poliomyelitic cords, although it is not as characteristic as the process just described. It is more often seen in advanced cases, in which meningeal infiltration and perivascular cuffing are maximal. In appearance the cells resemble closely the pathological forms described by Nissl as characteristic of ischemia. They display a thin and chromophobic

cytoplasm, with markedly contracted outlines. The nucleus stains dark blue with hematoxylin and eosin and tends to assume a characteristic triangular shape when cut in section. The nucleolus is swollen. The disposition of inorganic components in these cells provides an interesting contrast. The nucleus exhibits a heavy, powdery, refractile ash, while the cytoplasm is represented by a deposit so sparse that it can scarcely be appreciated in the incinerated specimen. It is evident that a migration of inorganic substance from the cytoplasm to the nucleus has taken place, an exact reversal of the process described above, in which the mineral matter of the cytoplasm increased at the expense of the nucleus.

DISCUSSION

As in other injuries and infections of the nervous system, edema occupies a conspicuous place in the pathology of poliomyelitis. It is usually accompanied by an acute swelling of the cells, which disappears as reaction to the injury subsides. Somewhat similar appearances may be produced by the intravenous injection of considerable quantities of water (Weed and McKibben ¹⁷). With a decrease in ionic concentration of the fluid environment of the cell one would expect to find a diffusion of water into the cytoplasm and a diffusion of salts outward. The slight diminution of cytoplasmic mineral constituents demonstrated by microincineration during the stage of swelling may thus be due both to loss of cytoplasmic salts and to dilution. The alteration may not be considered permanent, since it is but a stage in many diseases in which complete recovery ordinarily occurs, and it may be reversed by the injection of hypertonic solutions.

The earliest indication of severe injury is seen in the nucleus with the appearance of the bodies described by Covell ¹³ and by Hurst,¹⁴ who suggested that they might be specific for the disease. Such bodies have also been observed by Schultz ¹⁸ and by Stevenson,¹⁹ the latter author being more conservative in his interpretation, stating that they "seemed to be nothing more than a clumping of the intranuclear network." They appear in the drawings of Walter ²⁰ and of Wickman ²¹ who, however, did not mention them as being specific. Wolf and Orton ²² recently reported finding similar "intranuclear inclusions" in the spinal motor cells of patients dying of twenty-five

different diseases. The results of the present study indicate that they merely represent one stage in the total process of cellular degeneration. It is difficult to postulate the origin of the inclusion material. It might consist, as Wolf and Orton have suggested, of the vaguely defined substance known as "acidophilic chromatin," in which case it would represent an increase of a normal nuclear constituent. On the other hand, their mineral composition would indicate that these bodies represent an alteration of the nuclear ground substance by diffusion into it of some of the inorganic matter from the basophilic chromatin, which is gradually lost as its staining properties change from basophilia through amphophilia to acidophilia. Whatever it may be composed of, there is little doubt that the substance is formed with considerable rapidity and in large quantities, since it soon comes to fill the entire nucleus. The rate of its formation does not always parallel the rate of change in staining properties, a fact that may account for the occasional appearance of an acidophilic "inclusion body."

The granular stage of degeneration is one that has been overlooked by most investigators. Rissler²³ described a "granular clouding of the cytoplasm" as one of the earliest evidences of poliomyelitic injury, and Kraus and Gerlach²⁴ found certain deeply staining granules which they thought might be specific for poliomyelitis. Later, however, Gerlach and Kress²⁵ discovered them in other pathological states and decided that, although they were not unique to poliomyelitis, they certainly represented specific products of the action of a virus on a cell. They concluded that the granules were composed of lipofuscin (Lubarsch) or other similar pigment. In view of the fact that their inorganic content is high, and that most pigments are of almost purely organic composition, it seems unlikely that their conjecture could be true.

The frequent oversight of this stage of degeneration may be attributed to the briefness of its existence during the earliest stages of involvement (later stages are more often studied, especially in human cases) or to the difficulty of recognizing it in routinely stained sections. In the microincinerated preparations, on the other hand, the appearance of the granular cells is so striking that it cannot escape notice, and the change which renders them so obvious constitutes, from the microchemical standpoint, the most interesting phase of the entire process. Obviously, nuclear mineral matter enters the

cytoplasm but not in sufficient quantities to explain the increase. Some must be derived from without, from tissue fluid, blood or other sources by a necrobiotic attraction, a final vital action of the cell, after which it proceeds to complete disintegration. No explanation can be offered of the fact that some cells in this particular stage are phagocytosed, while others are not.

The recognition of the granules is important for another reason: it serves to classify the cytopathology of the disease. If the series of cell changes here described be compared with Spielmeyer's²⁶ account of Nissl's fundamental change, which he termed "*schwere Zellveränderung*," it will be found that they agree closely. If the same type of destruction has been observed in various diseases it would seem that the recognition of poliomyelitis by a single stage of the process would be impossible, and that the effects of the virus on the cell were anything but specific. The coincidental formation of inclusion bodies, which differ from the cell changes in other diseases showing this type of degeneration, would appear unlikely.

A problem of importance in any study of poliomyelitis injury is the determination of what might be called the threshold of mortality, or that amount of damage beyond which no recovery may occur. That some degree of normal function may return after the initial paralysis has long been known from clinical observations. O'Leary, Heinbecker and Bishop²⁷ showed that in the causation of paralysis we have to deal with some factor affecting the conductivity of the cell body. Covell²⁸ found that at the time their tests were made cell destruction was far advanced, so that the succession of cell changes described herein had probably already taken place. He also presented evidence that some of the partially destroyed cells might recover. The transient nature of acute cell swelling might suggest its relation to transient paralysis, but we know that this change may also occur in states in which no paralysis is present (meningitis, uremia, and so on). Unless, then, the paralysis is due to some specific action of the virus on the cell (which seems unlikely, considering the resemblance of the reaction to that seen in other diseases) we must conclude that it occurs some time after the appearance of nuclear changes. The results of this study indicate that the process of cell death is a complicated one. Osterhout²⁹ found the same to be true of planarians and was able to define a point beyond which complete recovery was impossible. Perhaps the same principle may be ap-

plied to nerve cells, in which case permanent loss of function would probably coincide with the stage of hypermineralization, since this stage, one would judge from the accounts of other authors, is approximately the time of destruction of the neurofibrillar network.

SUMMARY

The principal type of nerve cell destruction in poliomyelitis involves three stages: (1) edema, with acute swelling of the cell and diminution of its inorganic content; (2) granulation, with hypermineralization; and (3) acidophilic necrosis, with diminution of mineral constituents.

NOTE. The author is indebted to Dr. Gordon H. Scott and to Dr. E. V. Cowdry for help and encouragement.

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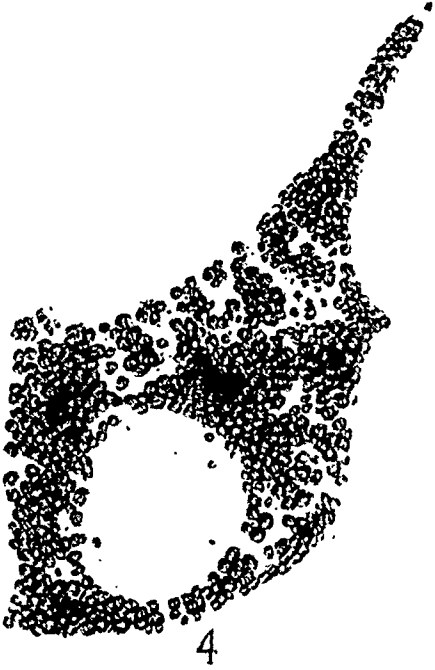
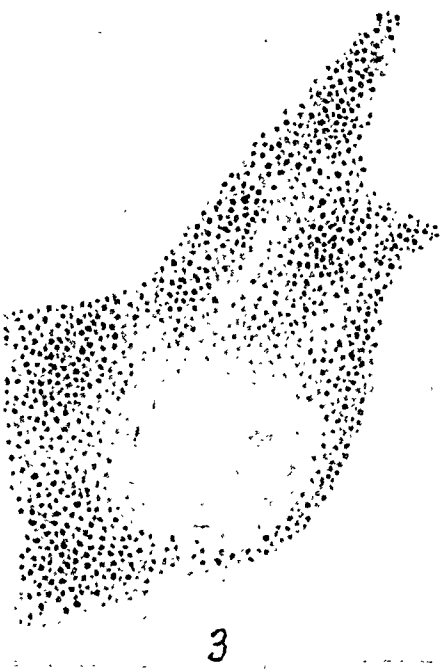
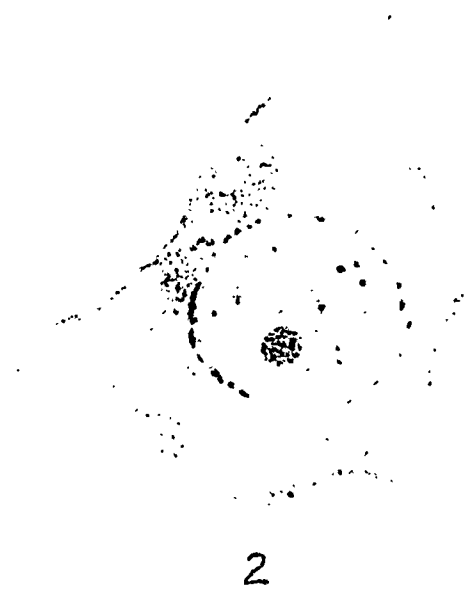
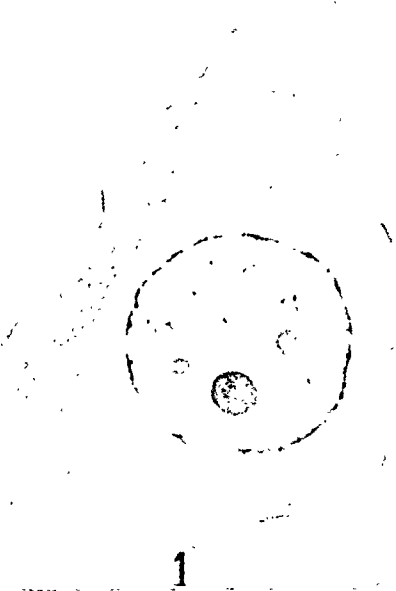
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DESCRIPTION OF PLATE

PLATE 143

All figures are camera lucida drawings of anterior horn cells from monkeys experimentally infected with poliomyelitis. The figures at the left are drawings of control sections stained with hematoxylin and eosin. The figures at the right are microincinerated sections of the same cell. Material figured was fixed in absolute alcohol-formalin.

- FIG. 1. Anterior horn cell from the cervical cord of a monkey experimentally infected with poliomyelitis showing acute swelling and the earliest stages of nuclear alteration. Two spherules of amphophilic material (the so-called inclusion bodies) are shown. The nuclear membrane is encrusted with basophilic chromatin, although some of this material still remains dispersed within the nucleus.
- FIG. 2. Microincinerated preparation of a serial section of the same cell showing diminished cytoplasmic ash and increased density of nuclear membrane.
- FIG. 3. Cell in the granular stage of degeneration. The nucleus is hyaline, acidophilic, and contains two round masses which take a deeper stain than the remainder of the nucleus.
- FIG. 4. Microincineration of a section of the same cell as pictured in Fig. 3 showing the extremely heavy ash deposit left by the cytoplasm. The nucleus at this stage is practically ash-free.
- FIG. 5. Acidophilic necrotic cell in which neuronophagia has not taken place. The cytoplasm exhibits a spongy texture and shows no cell membrane.
- FIG. 6. Microincinerated section of the cell shown in Fig. 5. The ash is webby in appearance and is glossy, though not highly refractile.



INTRANUCLEAR INCLUSIONS IN THE SALIVARY GLANDS OF MOLES *

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Intranuclear inclusions have been described in the salivary glands of guinea pigs, rats and humans, but only in guinea pigs has it been proved that they are caused by a filterable virus (Cole and Kuttner¹). We have noted² their presence in a fourth species, the common mole. This observation was made in the course of a brief survey, suggested by Dr. E. V. Cowdry, of the distribution of intranuclear inclusions in wild animals in the absence of distinctive clinical symptoms. In this paper we shall describe these inclusions in moles, mention our attempts to transmit a virus and compare the properties of the inclusions in the salivary glands of all four species.

MATERIAL

We list the other animals we examined, as well as the moles, because negative observations may be of some interest to investigators making more complete surveys. They were:

- 1 Evans king snake (*Lampropeltis calligaster*)
- 1 Red headed skink (*Eumeces quinquelineatus*)
- 1 Electric eel (*Electrophorus electricus*)
- 1 Bull frog (*Rana catesbeiana*)
- 2 Rats (*Mus decumanus*)
- 2 Mice (*Mus musculus*)
- 1 Gopher (*Spermophilus franklini*)
- 14 Moles (*Scalops aquaticus*)
- 1 Bat (*Myotis subulatus*)
- 1 Flying squirrel (*Glaucomys volans*)
- 4 Opossums (*Didelphis virginiana*)
- 11 Monkeys (*Pithecius rhesus*)
- 1 Gray cheeked mangabey (*Cercocebus albigena*)
- 1 Tapir (*Tapirus Indicus*)

* Aided by a grant from the Rockefeller Foundation for research in virus diseases.
Received for publication June 4, 1934.

Our thanks are due to Dr. A. M. Lucas for the first moles showing inclusions, and to Mr. George Vierheller, Director of the St. Louis Zoological Gardens, for the mangabey and tapir.

Careful autopsies were made of each animal and specimens were taken from a wide variety of tissues. Zenker and Zenker-formalin fixatives were used in all cases and the sections were colored with hematoxylin and eosin and by the Giemsa stain. Special microchemical tests were applied to the inclusions in the moles and attempts to transmit a virus were made which will be described later.

OBSERVATIONS

Figures 1-6 illustrate the appearance of the intranuclear inclusions in the salivary glands of moles. They were found in all of the 14 moles examined. Careful search in other tissues failed to reveal any. The moles weighed about 150 gm. and were presumably adults. The series did not contain any very young animals. The sections were 5 microns thick. On the average 1.8 inclusions were found per sq. mm. of section. The maximum number was 9 per sq. mm., while in 2 animals the inclusions were very rare, being seen only after intensive study of many serial sections.

The altered cells are usually situated in the terminal tubules of the serous portions of the glands and more rarely in the mucous parts (Fig. 5). None occur in cells that can be definitely classified as duct cells but they are rather more numerous near the ducts. Many appear to be shoved off into the lumens of the tubules (Fig. 6).

An accompanying tissue reaction was noted in 8 animals. It consisted of a slight perivascular and interstitial lymphocytic infiltration. But the extent of the infiltration was not proportional to the number of affected secretory cells. Neither did the location always correspond. In some cases many cells possessing inclusions were observed in the absence of infiltration. Two nematodes were noted. The first, belonging to the genus *Capillaria*, was found in the epithelium of the tongue of 6 moles; and the second, apparently a member of the genus *Porrocaecum*, was observed in the salivary glands of 7 moles. For the identification of both of these we are indebted to Dr. E. W. Price of the Bureau of Animal Industry. There was no sharp correlation between the incidence of the parasites and the inclusions, but it was noted that in several sections the number of inclusions was

definitely higher near the *Porrocaeca*. The parasites were usually well walled off by connective tissue and were frequently accompanied by a mild degree of cellular infiltration, consisting chiefly of lymphocytes. The tissue reaction seemed to bear no constant relation to either the inclusions or parasites. In one mole there was, as might be expected, a heavy accumulation of eosinophile leukocytes near a *Porrocaecum*.

An outstanding feature of the inclusion-containing cells and nuclei was marked hypertrophy. The diameter of the altered cells averaged about 15 microns — approximately $1\frac{1}{2}$ times the diameter of the normal cells. The extent of enlargement for both serous and mucous cells was remarkably uniform, as indicated in Table I.

TABLE I

Measurements in Microns of Inclusion-containing Cells in the Salivary Glands of Moles

Cells	Diameter measured	Range	Mean	Median	Mean deviation on mean	Standard deviation
200 inclusion-laden cells in the mixed portion of the gland	Cellular	10.00	15.37	15.06	1.57	2.13
	Nuclear	9.34	10.14	10.67	1.56	1.83
	Inclusion	6.88	6.12	5.69	1.01	1.29
25 inclusion-laden cells in the mucous portion	Cellular	8.13	16.85	16.77	2.03	2.34
	Nuclear	6.25	12.05	12.03	1.75	2.13
	Inclusion	2.50	7.05	6.36	0.81	0.98

The mean is the mathematical average of the measurements obtained by dividing the sum of the diameters by the number. The median is the middle number in the series of numbers arranged in the order of magnitude. The fact that the mean and median are so nearly the same indicates a central tendency. However, one can easily imagine that a distribution in which there is an equal clumping at each end of the range might easily give a median and a mean of almost equal value. To minimize this possibility of error the "mean deviation on the mean" and the "standard deviation" were calculated. In the "mean deviation on the mean" we have a figure showing the average distance of all members of the series from the mean, or average, of the series. This is remarkably small and gives a truer indication of a central tendency. The "standard deviation" is

a measurement which, when marked off on both sides of the mean, will include two-thirds of the cases involved, when the cases have been arranged in the order of magnitude. Interpretation of this central tendency is difficult. It may be that the cellular response, whatever its nature, has reached a kind of end point characterized by relative uniformity in degree of hypertrophy. It is possible also that the early stages of hypertrophy, so rare in our specimens, seem to be lacking because they may be passed through quite rapidly; and that final stages of further hypertrophy or disintegration are absent, because the cells may be cast out through the ducts at this particular stage. Moreover, the infrequency of early stages may indicate that the process is no longer an active one.

The intranuclear inclusions themselves show a parallel uniformity in size. The range from the largest to the smallest was slight. Occasionally they seemed to be paired, or subdivided (Figs. 2 and 4). Their outlines were somewhat irregular. The centers were denser than the periphery. They were definitely basophilic, only rarely showing any tendency towards acidophilia — a property that we do not think can be attributed to unusual fixation or some deviation from standard technique.

The intranuclear inclusions exhibit marked Feulgen and masked iron reactions. Mucicarmin and Millon's reagent gave inconclusive results. Margination of chromatin on the nuclear membrane was not very noticeable. This may be correlated with the retention by the inclusion of basophilic properties (thymonucleic acid and masked iron). The nucleolus was recognizable in some cases either applied to the inclusion or between the latter and the nuclear membrane. A clear thin halo of chromophobic nuclear material is invariably interposed between the inclusion and the nuclear membrane and is very typical.

In the cytoplasm of most cells 10 to 30 small, basophilic inclusions were clearly visible, closely resembling those described in detail in the guinea pig's submaxillary glands by Pearson.³ They accompanied approximately 40 per cent of the nuclear inclusions and occurred in both serous and mucous types (Figs. 1-5).

Many attempts were made to demonstrate the existence of an active virus in the salivary glands, but saline emulsions injected intracerebrally, subcutaneously and intraglandularly into rats, mice, rabbits and young guinea pigs proved futile (Table II). The animals

that died in a few hours did so as a direct result of injury to the brain when the injection was made and not from the action of a virus. Those that survived until they were sacrificed showed no signs of dis-

TABLE II
Attempts to Pass Virus

Mole No.	Inoculum (saline emulsion)	Recipient animal	Injection	Result
4	Fresh tissue	Rabbit	Intracerebral	Died — 6 hours
4	" "	Young guinea pig	Subcutaneous	Killed — 30 days
5	" "	Rabbit	Intracerebral	Died — 6 hours
5	" "	"	"	Died — 12 hours
5	" "	"	"	Killed — 14 days
5	" "	Young guinea pig	"	Died — 12 hours
4, 5, 6	Glycerin preserved tissue	White mouse	"	Killed — 35 days
4, 5, 6	" " "	" "	"	Died — 3 days
4, 5, 6	" " "	" "	"	Killed — 38 days
4, 5, 6	" " "	White rat	"	39 days
4, 5, 6	" " "	" "	"	39 days
4, 5, 6	" " "	" "	"	39 days
4, 5, 6	" " "	Young guinea pig	"	38 days
4, 5, 6	" " "	" " "	"	38 days
4, 5, 6	" " "	" " "	Submaxillary gland	20 days
12	Fresh tissue	" " "	Intracerebral	Died — 24 hours
12	" "	" " "	"	Died — 12 hours
12	" "	" " "	"	Killed — 62 days

ease. Histological examination of the tissues at the site of injection revealed no significant alterations.

A detailed comparison of these intranuclear inclusions in moles with our own preparations of inclusions in guinea pigs, with speci-

mens showing inclusions in human salivary glands and in rats sent to Dr. E. V. Cowdry by Dr. S. B. Wolbach and by Dr. Juanita Thompson respectively, together with a close study of the literature, brings to light a number of interesting features.

1. The inclusion incidence of 100 per cent of 14 in moles is to be compared with 12 per cent of 183 in humans (Farber and Wolbach⁴), 84 per cent of 75 in guinea pigs (Cole and Kuttner¹), and 14 per cent of 70 in rats (Thompson⁵).

2. It is not feasible to compare the number of inclusion-containing cells per unit volume of tissue because the observations on species other than the mole are not quantitative. Probably they are equally numerous in the mole, as in the others.

3. The location of the inclusions in moles is in the serous and mucous gland cells and not in the duct cells, whereas in the others the duct cells are often more frequently affected than the gland cells.

4. In the mole, guinea pig and rat the occurrence of inclusions is apparently limited to the salivary glands, but in humans intranuclear inclusions have been found in other tissues of the same individuals (see Farber and Wolbach⁴ and their review of literature).

5. The size of the hypertrophied cells is greatest in humans, about the same in guinea pigs and rats and least in the moles.

6. The morphology of the inclusions is similar in all four. Their outlines are irregular, often their central parts are denser. From the periphery strands of material may extend toward the nuclear membrane. The inclusions are not evenly rounded, spherical droplets of material, nor are they hyaline in appearance like Cowdry's type B inclusions.⁶

7. The inclusions are apparently more basophilic in the moles than in any of the others. They also yield a more marked Feulgen reaction for thymonucleic acid and Bensley-Macallum test for masked iron than Cowdry⁷ secured with the guinea pig inclusions. Data on thymonucleic acid and masked iron are not available for the human and rat inclusions.

8. Margination of basophilic chromatin on the nuclear membrane is absent or slight in moles — a circumstance that may be related to the retention of basophilia by the inclusions. This margination is very conspicuous in guinea pigs and comparatively slight in humans and rats.

9. The nucleoli behave in the same way in all four species in so far that they do not contribute noticeably toward the formation of the inclusions.

10. A clear, unstaining halo between the inclusion and the nuclear membrane is most noticeable in the guinea pig, less so in the mole, and least, but to about the same degree, in humans and rats.

11. Similar basophilic bodies occur in the cytoplasm of the inclusion-laden cells in all four species. In the mole, however, they are found on all sides of the nucleus and apparently do not show quite the same tendency to be clumped in the distal cytoplasm, between the nucleus and lumen, as in rats, guinea pigs and humans.

12. No constant accompanying reaction or degeneration of the tissue about the affected cells is found in any of the forms, but, in the case of all of them, mention is made of occasional infiltration by lymphocytes and phagocytic cells.

13. No parasites like those herein reported in the oral epithelium and salivary glands of moles, or of any sort, have been described in humans, guinea pigs or rats.

14. Detection of the inclusions in these four species has been to some extent a matter of chance. In none of them is attention drawn to the inclusions by the exhibition of definite clinical symptoms.

DISCUSSION

The intranuclear inclusions in the salivary glands of moles are sufficiently like those in humans, guinea pigs and rats to suggest a more or less common origin. Only in the guinea pig has a virus been identified as the etiological factor, but the opportunity to demonstrate virus action in the others has not been favorable. It is likely that systematic study of the salivary glands in many species will bring to light further instances of the occurrence of similar inclusions in the absence of distinctive signs of disease. The fact that the intranuclear inclusions in this location are so very large does not of itself indicate that the causative agents if, as in guinea pigs, they turn out to be viruses, are peculiar or very different from those that influence other tissues. When the submaxillary virus of guinea pigs is led to act on the brain it produces inclusions that are not particularly large in cells that are not hypertrophied. Consequently it may be supposed that at least two factors are involved — the nature of the virus and the reactivity of the particular cells.

SUMMARY

All of 14 moles examined show, in the absence of clinical symptoms, intranuclear inclusions in their salivary glands which resemble in many respects those previously reported in humans, guinea pigs and rats.

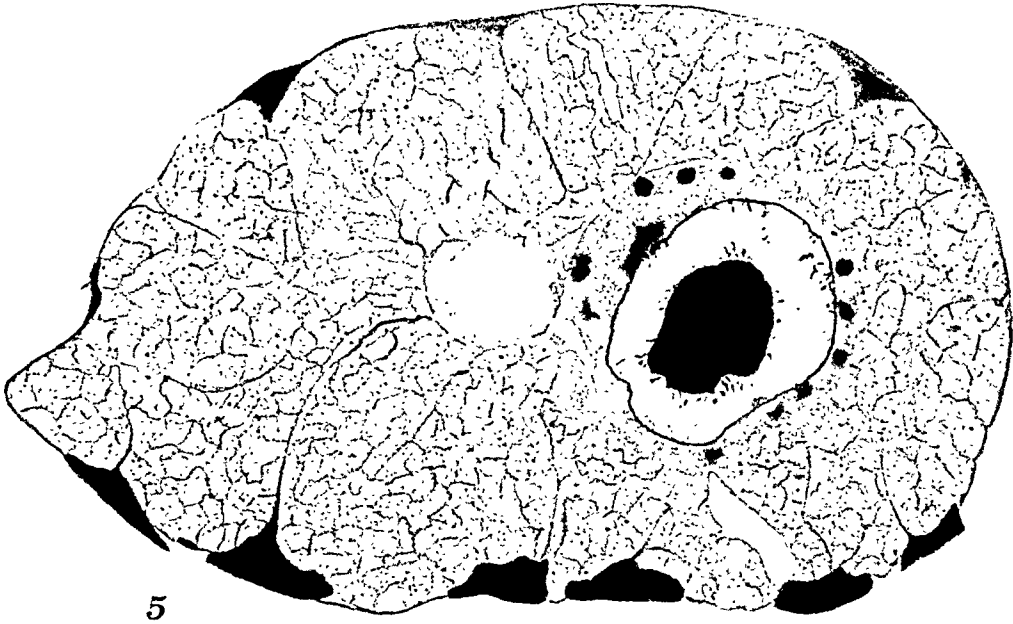
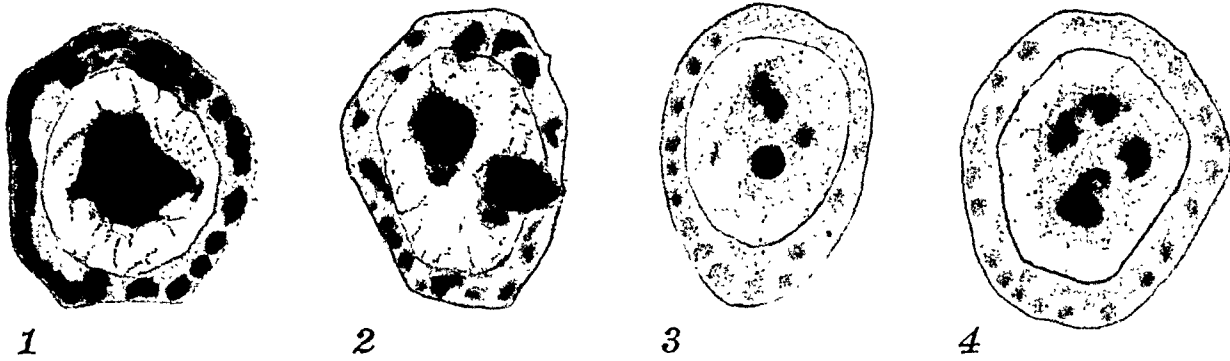
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DESCRIPTION OF PLATE

PLATE 144

- FIGS. 1 to 4 inclusive. Drawings of affected cells to show detailed structure of intranuclear inclusions and position of cytoplasmic inclusions. Note the paired inclusion in Fig. 2 and the presence of the nucleolus in Fig. 3. $\times 2300$.
- FIG. 5. Drawing of mucous acinus to show relative size and position of the affected cell. Note the encroachment upon the lumen. $\times 2300$.
- FIG. 6. Drawing of mucous and serous acini showing involved cells in which cytoplasmic inclusions are absent. $\times 1300$.



Rector and Rector

Intranuclear Inclusions in Salivary Glands

ENCEPHALOMYELITIS, PROBABLY DUE TO LEAD POISONING *

REPORT OF A CASE

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Numerous cases of accidental lead encephalitis in man, as well as experimental lead intoxication in animals, have been reported in the literature. Kato ¹ in 1932 published a complete review of the Japanese literature on this subject and added many new cases of his own. But, in spite of the extensive investigations with animals and the careful study of human material, an agreement as to the pathological lesions in this type of cerebral involvement is still lacking. Friedländer ² found no alterations in the brain in his case, while Cadwalader, ³ Lehmann and co-workers, ⁴ and McCarthy ⁵ reported striking changes in the nerve cells in the brains of both humans and animals dead of lead poisoning.

Lehmann and his co-workers experimented with cats and caused a "liquefaction" of the nerve cells with a destruction of both nucleus and cell body. McCarthy produced cerebral lesions in dogs with lead acetate and noted an extensive chromatolysis and vacuolization of the nerve cells with a slight glial proliferation. Somewhat similar findings were reported by Cadwalader in human material. Hassin, ⁶ however, in 3 cases found the nerve cells intact. He noted only thickening of the pia arachnoid and slight glial proliferation. Barron and Habein ⁷ reported a case of lead encephalitis in which the brain was histologically normal. McKhann and Vogt ⁸ in a recent publication suggested the possibility that much of the cerebral symptomatology in lead encephalitis might be due to an increased intracranial pressure and not to an actual destruction of nerve cells.

Spinal cord lesions from lead poisoning seem to be even less frequent than the cerebral involvement. Bechtold, ⁹ Sons, ¹⁰ Lewin and Treu ¹¹ and Propper ¹² have published complete clinical reports of

* Received for publication March 22, 1934.

lead myelitis with recovery, while Spiller¹² and Cadwalader have described the histological changes in the spinal cords of their cases. Spiller found an extensive degeneration of the anterior horn cells with no alteration in the white matter. In Cadwalader's case the pathological findings closely resembled those of amyotrophic lateral sclerosis. The anterior horn cells were severely injured and there was a mild degeneration of the lateral columns. Only a few fibers were destroyed in the posterior columns and these were noted only in the lumbar segments.

In view of the absence of detailed pathological reports dealing with severe lead encephalomyelitis a full report of the following case seems warranted.

REPORT OF CASE

Clinical History: J.X. J., a male aged 53 years, was first admitted to the hospital Sept. 22, 1930, complaining of increasing weakness and loss of weight. In January, 1930, he had secured employment, which consisted of dipping metal parts into a tank of lead paint. While working, his forearms and arms were continuously immersed in the paint, which also splashed freely over his neck and face. He felt well until the first part of June, when he began to notice a taste of paint in his mouth and had periodic attacks of nausea and vomiting without any associated abdominal cramps. Early in July an intense skin irritation of the upper extremities developed which was diagnosed as "paint dermatitis" and proved extremely resistant to local treatment. He continued working until August 15th, although gradually losing weight and strength. At the time of his first admission to the hospital he had lost 14 pounds in weight.

The past history was essentially negative. While working in a boiler factory in 1917 his hearing had been impaired. There had never been any gastrointestinal disturbances and, until the onset of his present illness, the patient's weight had remained constant, at about 150 pounds. His father died at the age of 62 years from a carcinoma of the stomach. His mother died at 23 years of age from tuberculosis. He had been married 29 years and his wife is living and in fairly good health.

Physical Examination: The patient appeared anemic; he was quite emaciated and had a sallow, yellowish complexion. There was a grayish discoloration of the hands and forearms with many excoriated lesions scattered over these areas. The pupils were equal and regular and reacted normally to light and accommodation. The ocular movements were unimpaired. There was a marked gingivitis and extensive dental caries but no definite lead line. The tongue showed no atrophy. The lungs were normal. The heart was normal in size, shape and position; rate 76, no murmurs, tones normal. The blood pressure was 142/86. Abdominal examination was negative.

Neurological Examination: The cranial nerves were normal except for a partial nerve deafness in both ears. The abdominal reflexes were decreased but all the deep reflexes were fairly normal. Vibration sense was diminished in the ankles and legs, being almost completely absent on the right side. There was no

sign of ankle or wrist drop or of any other muscular weakness. At this examination the neurologist concluded that the findings were insufficient to warrant a positive diagnosis of any organic disturbance of the central nervous system.

The patient remained in the hospital for 19 days and, after his discharge, reported to the outpatient department for further observation and study. Throughout this period numerous laboratory studies were performed. The urinalysis was negative on four occasions. The icterus index was 12 units. Gastric expression revealed no free hydrochloric or lactic acid but contained 10 degrees of total acidity at 10 minutes, and 20 degrees at 20 minutes. The stools contained no pus, mucus or occult blood, and microscopic examination was negative for ova and parasites. The Wassermann test was repeatedly negative, as was also the spinal fluid examination. Roentgen-ray studies of the chest and abdomen gave normal findings. The blood studies are tabulated in Table I.

TABLE I
Examinations of the Blood

Date	Red cell count	Hemoglobin	Color Index	White cell count	Reticulocytes
		<i>per cent</i>			<i>per cent</i>
9/19/30	2,670,000	82	1.6	6750	
9/21/30	2,840,000	79	1.4	7300	
9/29/30	2,000,000	70	1.7	7000	20.0
10/ 1/30	2,630,000	66	1.2		
10/ 7/30	2,520,000	67	1.3	4850	1.0
10/ 9/30	2,340,000	65	1.4	6600	1.8
10/10/30	2,260,000	62	1.4	6400	1.3
10/11/30	2,010,000	60	1.5	6150	2.0
10/14/30	2,440,000	61	1.3	6650	1.4
10/18/30	2,150,000	60	1.4	8050	2.0
10/22/30	2,130,000	57	1.3	6300	2.8
10/24/30	2,470,000	47	0.95	6850	7.5
10/29/30	2,040,000	59	1.6	7600	3.3

The blood smears were studied repeatedly by the department of hematology and were described as follows. "The red cells show the following features: anisocytosis (macrocytes, normocytes and microcytes), poikilocytosis, hyperchromasia, occasional polychromatophilic cells and occasional stippled cells (the basophilic stippling was increased after administration of potassium iodide). The neutrophil leukocytes are present in normal numbers but are toxic, their nuclei showing a marked 'shift to the right.' These cells were given careful study because the neutrophils of pernicious anemia usually show a similar change, but these cells are not of the pernicious anemia type, since their nuclear lobes are too plump and their neutrophil granules are not of the right type. It may be concluded that the patient's anemia is of a toxic type and compatible with lead poisoning but not characteristic of pernicious anemia."

After the patient's discharge from the hospital his symptoms gradually became augmented. An attempt was made to de-lead him with potassium iodide, but this medication caused a definite exacerbation of symptoms, a marked increase in the basophilic stippling of the red cells and a drop in the hemoglobin of

13 per cent in a period of 6 days (60 per cent to 47 per cent). A milk diet was then prescribed and calcium lactate given in large doses by mouth. On this type of therapy, which tends to keep the lead stored in the bones, he promptly improved and the hemoglobin increased 12 per cent in the next few days. This experience in treatment seems to us to be of definite diagnostic significance.

Throughout his illness the hemoglobin remained at about 50 per cent and resisted all attempts at correction. Liver extract was given orally over a long period with no results, although three injections of liver intramuscularly were followed by a temporary rise in the hemoglobin, the red cells remaining low.

By August, 1932, nearly 2 years after the onset of the ailment, weakness had become so marked that it greatly interfered with walking. A definite spasticity of the lower extremities had also developed. The weakness and spasticity advanced rapidly, so that by January, 1933, the patient could no longer walk alone and was confined to bed, where he remained until death. A neurological examination was performed in November, 1932, at which time the patient was so weak that he was unable to walk alone. All voluntary movements of the arms and legs could be executed, although he was manifestly ataxic. None of the deep reflexes was elicited even with reinforcement. The position and vibration senses were entirely absent in all extremities, while the deep tendon and muscle sensations appeared only slightly impaired. Superficial sensibility was intact. The findings at this examination seemed compatible with a subacute combined degeneration of the cord.

During the last 6 to 8 months of life definite signs of mental involvement developed. He would become disoriented and talk irrationally for hours. Terminally a urinary retention developed. He died on June 27, 1933, after having been bedridden for 6 months.

POSTMORTEM EXAMINATION

The body was that of a poorly nourished, elderly male, 161 cm. long, weighing about 110 pounds. There appeared to be moderate atrophy of the muscles of the upper extremities but this may have been due to the extreme degree of undernourishment. The abdominal muscles were well developed. A large decubitus was present over the sacrum.

The peritoneal cavity showed nothing of note. The right pleural cavity, anteriorly and inferiorly, was obliterated by dense fibrous adhesions; the left pleural cavity as well as the pericardial sac was normal.

The heart weighed 200 gm. and was normal. The coronaries were sclerotic and their lumens were very much narrowed in places, although at no point completely closed. Each lung weighed 300 gm. and contained a moderate amount of edema and congestion but no free pus. The spleen weighed 200 gm. and showed no disease.

The liver weighed 1300 gm. Its surface was smooth. On section

the interlobular spaces were more conspicuous than normal and there was a slight yellowish tinge to the parenchyma. The gall-bladder and ducts were all normal. The gastro-intestinal tract, pancreas, adrenals and kidneys showed no disease.

MICROSCOPIC EXAMINATION

Microscopic examination of the organs confirms the gross impressions. The liver shows a moderate fatty metamorphosis and, with special stains, no hemosiderin can be detected in the liver tissue.

The brain and spinal cord were carefully removed and immediately fixed for special staining. In gross the brain showed nothing of note but the entire cord was unusually small. The dura mater and pia arachnoid appeared normal, not showing the thickening often mentioned by other investigators.

Sections were taken from several cortical areas, the basal ganglia, midbrain, cerebellum, medulla oblongata and various levels of the spinal cord. These were stained in the following ways: hematoxylin-eosin, Weigert's myelin sheath stain, iron hematoxylin-Van Gieson, Nissel's method, Bielschowsky's stain, and sudan III.

Throughout the cerebral cortex there is a marked shrinking of the ganglion cells, leaving a large clear space about the remains of each. In certain of these cells the dendrites are intact, in some scarce or absent, while in others they are fragmented and separated from the cell body. In many cells the cytoplasm is almost entirely absent, leaving only a nucleus situated in a large clear area; in others the nucleus is surrounded by an irregular border of cytoplasmic substance (Fig. 1). The nuclei of the cortical ganglion cells are usually intact and occupy a central or exceptionally a peripheral position. Occasionally the nuclei are shrunken, deeply staining, and separated from the cytoplasm by a zone of varying width (Fig. 1). Nucleoli are usually present. In some areas of the brain, chiefly the temporal lobe and the basal nuclei, another type of change is noted. Here the ganglion cells are swollen to almost twice normal size; their cytoplasm is homogeneous and glossy and the processes are usually absent, giving the cells a rounded edematous appearance (Fig. 2). The nuclei of these large cells are still intact but many have irregular edges and stain poorly. Careful study of the sections reveals all stages from the

slightly swollen cells to those exhibiting complete homogeneity and disintegration. With the special Nissl stain it is readily apparent that many cells have almost completely lost the Nissl substance (Fig. 2). There is only slight indication of neuronophagia in spite of the partial destruction of the nerve cells. Satellites are absent.

Sections from the floor of the fourth ventricle reveal many small hemorrhages, chiefly of a perivascular distribution. The brain tissue around these hemorrhages does not seem to be injured. There is no cellular infiltration or injury to the white matter.

In contrast to the brain the most constant damage to the spinal cord is in the white substance, the gray matter, as a rule, remaining free from injury.

The cervical cord presents the most marked changes. With the Weigert stain a complete demyelination is noted in the columns of Goll and Burdach, with a partial involvement of the dorsal and ventral spinocerebellar tracts, and of the lateral and ventral corticospinal tracts (crossed and direct pyramidal) (Fig. 3). The direct pyramidal tract presents an asymmetrical involvement showing a more extensive demyelination on the right side of the cord where the destruction extends into Marie's column (fasciculus sulco-marginalis). With the hematoxylin-eosin and iron hematoxylin stains many cells are detected invading the degenerated white matter. These cells contain a nucleus which is polymorphic and deep staining and a cell body that either is quite without definition or is fairly large, granular and irregular. A fat stain proves these cells to be macrophages in the various stages of development.

The gray matter presents but few alterations. A few of the anterior horn cells are shrunk, although none show any chromatolysis and there does not appear to be a decrease in their number. Numerous dilated blood vessels are scattered throughout the gray substance but no hemorrhages, either diffuse or perivascular, can be detected. The anterior and posterior rootlets are normal, showing no demyelination and no involvement of their axis cylinders. There are no signs of any inflammation (Fig. 3).

In the midthoracic cord the changes are almost the same as in the cervical region except that the demyelination is less in the medial parts of the posterior columns. There is still an involvement of the medial and ventral parts of the anterior columns of white matter, in spite of the fact that, at this level, the direct pyramidal tract usually

has decussated and therefore is absent. The anterior and posterior horn cells appear normal, both in number and in structure. In the nucleus dorsalis (Clark's column) the cells are greatly decreased. The few that are present have no processes and appear rounded, with smooth edges, and are diminished in size. Their nuclei are, as a rule, absent. The rootlets are unaffected and the arteries are normal.

The lumbar cord presents the least involvement (Fig. 4). There is only a partial destruction of the posterior columns and the crossed pyramidal tracts. The marked macrophage reaction is still seen in the demyelinated areas. The anterior horn cells and the rootlets are unaffected.

The appearance of the cord with the fat stain is similar in all the destroyed areas. All stages of transformation in the macrophages can be observed, from cells that contain only a few fat granules to others that are completely filled. These cells vary in appearance from small uniform ones with a central nucleus to large irregular cells in which the nucleus is either forced to one side or entirely invisible because of the large fat droplets. Often the macrophages clump together, presenting the appearance of a large mass of fat globules with an occasional small dark nucleus visible in the fatty mass. There is a great tendency for these cells to migrate to the blood vessels, all the vessels in the demyelinated areas being surrounded by collars of fat-filled cells. In many of the vessels the macrophages must have penetrated the walls, the lumens being completely filled with fat.

DISCUSSION

The pathological study clearly indicates that the lesions in our case are almost entirely degenerative in nature and differ greatly in the various parts of the central nervous system. In the brain the ganglion cells of the gray substance are involved, while in the spinal cord most of the damage is in the white substance, giving the appearance of a combined degeneration. Such a degenerative process is usually considered as a primary alteration, due to the selective toxic influence of the lead on the various parts of the nervous system, but the reason for the great difference in localization of the destructive process in the central nervous system is at present unknown.

SUMMARY

1. A case of encephalomyelitis is reported in a male who was thoroughly exposed to lead paint for a period of 7 months.
2. A pathological study of the nervous system revealed a marked destruction of the nerve cells in the cerebral cortex and an extensive demyelination of various tracts in the spinal cord.
3. The literature dealing with lead encephalomyelitis is reviewed.

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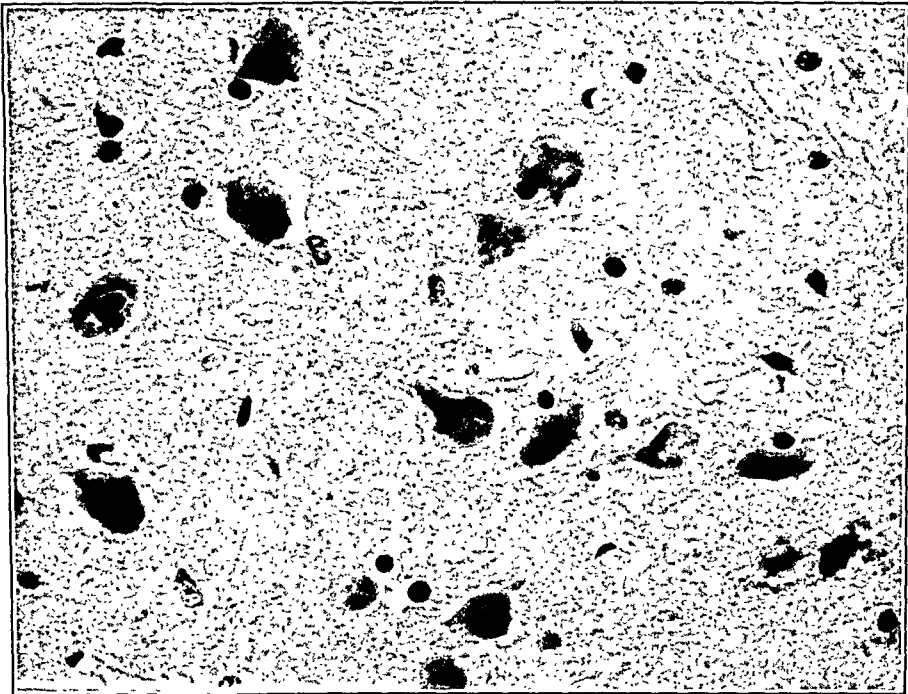
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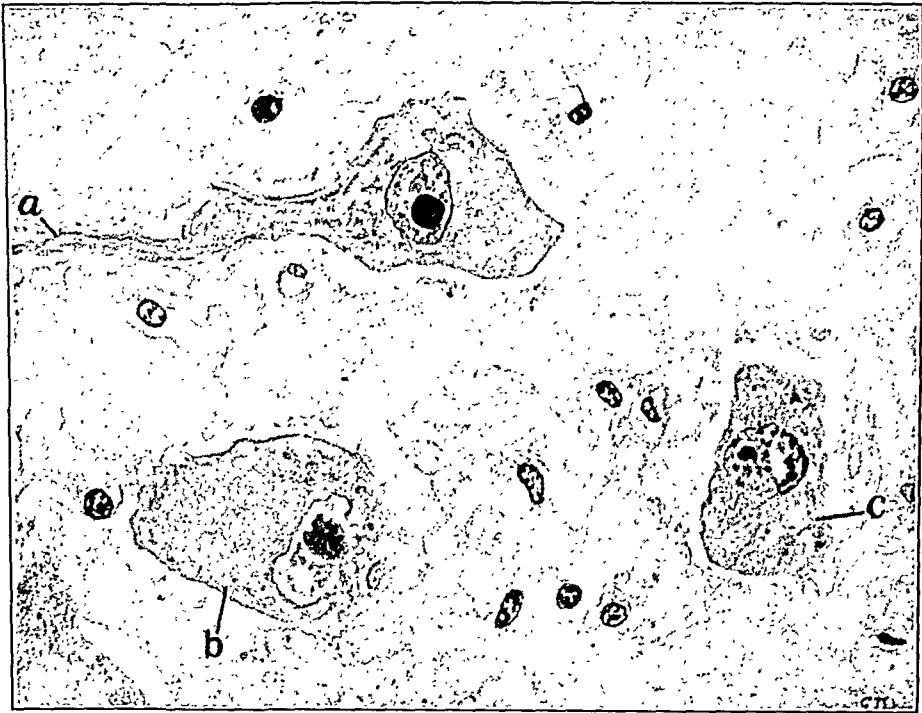
DESCRIPTION OF PLATES

PLATE 145

- FIG. 1. Section through the cerebral cortex showing the marked shrinking and irregularity of the nerve cells.
- FIG. 2. Drawing showing some of the ganglion cells of the temporal cortex. All the cells are swollen and present a marked chromatolysis. The cell marked "a" is an axone. In cells "b" and "c" there is also a destruction of the processes, giving the cells a rounded appearance.



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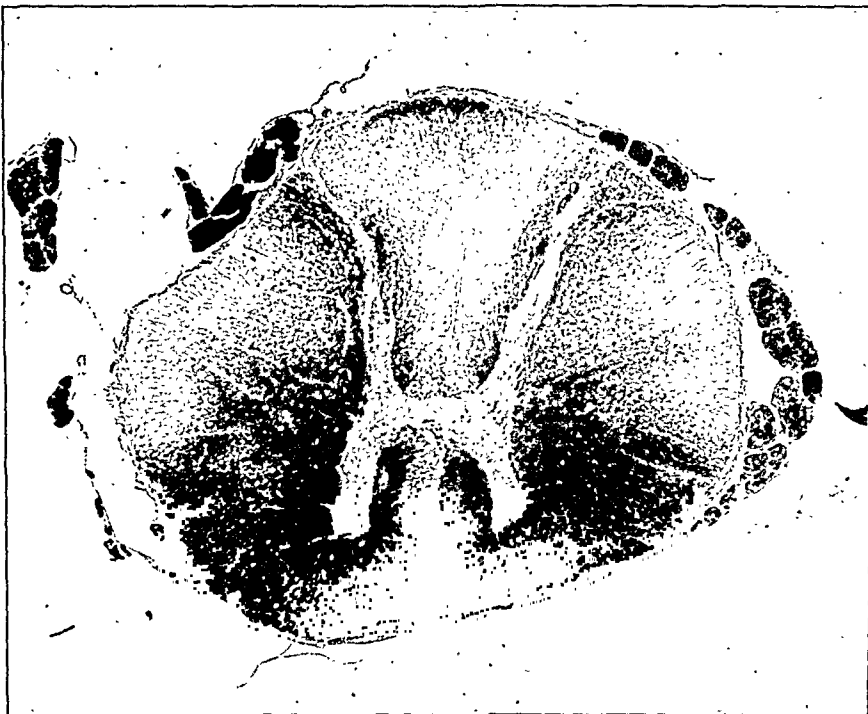


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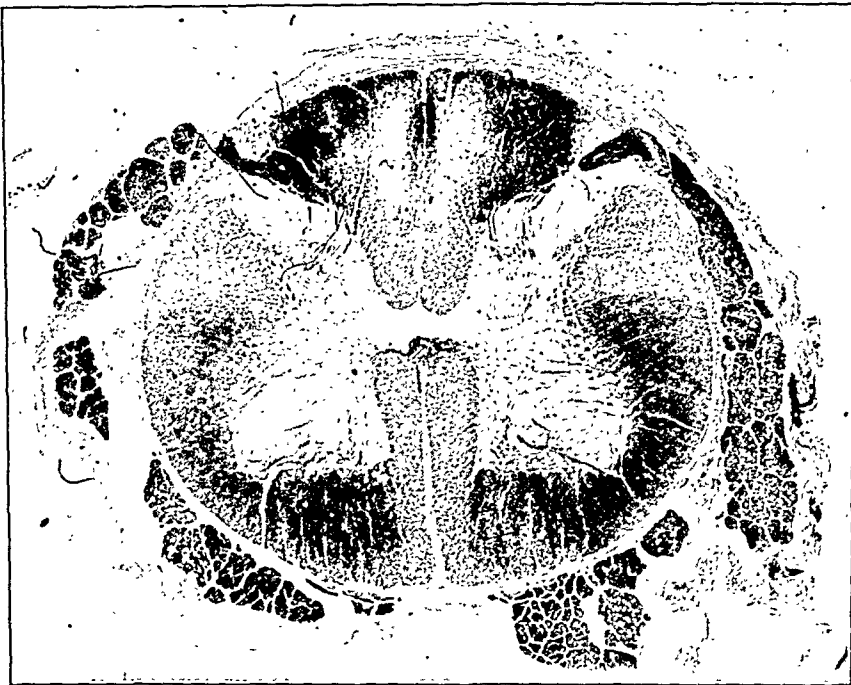
PLATE 146

FIG. 3. Section through the cervical cord showing the striking demyelination of the posterior, the lateral, and part of the anterior columns. The rootlets are intact.

FIG. 4. Section through the lumbar cord. There is only a partial destruction of the posterior columns and the crossed pyramidal tracts.



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4

UNIDENTIFIED PARASITE IN HEART MUSCLE *

WILLIAM C. VONGLAHN, M.D.

*(From the Department of Pathology, College of Physicians and Surgeons,
Columbia University, New York City)*

In the routine study of heart muscle from a case of aortic stenosis and insufficiency peculiar bodies were discovered in the myocardium. Since a thorough search of the literature failed to reveal any record of similar bodies having been described in the heart it seemed advisable to report the case.

The patient (History No. 316,149, Autopsy No. 10,821), a carpenter, aged 63 years, born in Virginia, was first admitted to the Medical Service of the Presbyterian Hospital because of cardiac decompensation. He was found to have aortic stenosis and insufficiency. At the end of 4 weeks the cardiac condition was sufficiently improved to permit him to return to his home. That evening he had a chill and came back to the hospital. It was determined that he had lobar pneumonia due to pneumococcus type II. He died on the 4th day of this illness.

At autopsy the heart was found to be hypertrophied, weighing 500 gm. The aortic valve was extensively calcified. The middle and lower lobes of the right lung were consolidated.

Peculiar solid bodies were found lying within the sarcoplasm of the hypertrophied heart muscle. The end of the body adjacent to the nucleus was bluntly rounded, the other end pointed. Near the bluntly rounded end was an oval vesicular nucleus containing one or more chromatin particles, and close to this nucleus, in some instances, a solid round structure. One or more oval vacuoles were present, often near the pointed end. The bodies averaged 52.5 microns in length and 5.5 microns in width. They were usually straight, except for slight undulation. In Figure 1 is shown a photographic reconstruction of one of these structures. One body was found that was turned abruptly at right angles close to the muscle nucleus, another was sharply bent upon itself.

* Received for publication May 24, 1934.

Another of these bodies was divided longitudinally through part of its length, so that it was roughly Y-shaped. There were two rounded ends directed toward the nucleus; the other end was sharply pointed. In one of the limbs with the rounded ends were two nuclei, in the other, a single nucleus. A large vacuole was present just where the two limbs joined.

Two larger bodies were discovered that had been cut across in sectioning the block of muscle. One of these fragments measured 63 microns in length and 7 microns in width; near the rounded end was a huge nucleus 5.8 microns wide in which were three nucleoli, the largest 3.8 microns in diameter. The nucleus was constricted at its midportion (Figs. 2 and 3).

The fragment of the second body likewise had a rounded end; it measured 45.5 microns in length and 7 microns in width. The oval nucleus was 10.5 by 4.5 microns. The single nucleolus was 2.4 microns in diameter (Fig. 4).

In many of the muscle fibers fragments were found and it was obvious that they were parts of more than one of these bodies.

The staining reactions were as follows.

STAIN	BODIES
Hematoxylin-eosin	Eosinophilic
Gram	Gram-positive
Mallory's phosphotungstic acid hematoxylin	Yellow
Heidenhain's iron hematoxylin	Black
Carbol fuchsin followed by 1% acid alcohol	Not acid-fast
Giemsa	Nucleus deep blue, remainder robin's egg blue
Eosin-methylene blue, azur B	Nucleus very deep blue, remainder dark blue

The only internal structure that could be distinguished in these bodies, aside from vacuoles and nucleus with its nucleolus and chromatin material, were fine granules in the bluntly rounded portion. In the phosphotungstic acid hematoxylin preparations the peripheries of the bodies were refractive.

These bodies were not encapsulated; they seemed somewhat rigid in the fixed preparation. They appeared to be surrounded by clear fluid. They were situated always adjacent to the muscle nucleus with the long axis parallel to the direction of the myofibrils. The nucleus of the muscle was always indented or invaginated by the

bodies. The myofibrils were pushed to either side by the bodies, but were otherwise unchanged.

The heart muscle containing these bodies was not enlarged, as compared with adjacent muscle that had none of them. There was no inflammatory reaction about muscle containing the bodies.

The bodies were very numerous in the posterior part of the left ventricle and were less frequently observed in the muscle from the apex of this ventricle. None could be found elsewhere in the heart. The only voluntary muscle available for histological study was the diaphragm; none of these bodies could be discovered in it.

These bodies do not conform to any known form of degeneration. Their definite shape and well preserved nuclei, together with their staining reactions, would indicate that they are not simple degeneration products. They do not resemble any hitherto described parasite known to lodge in heart or voluntary muscle.

Preparations have been shown to many pathologists and protozoologists. The consensus of their opinion was that these bodies were of parasitic nature but none could identify them. It is not believed that the bodies had any part in the production of the hypertrophy of the heart.

DESCRIPTION OF PLATES

PLATE 147

FIG. 1. Photographic reconstruction of a body within a single muscle fiber. Due to slight undulation the entire body could not be brought into focus; it was photographed in segments and these matched as accurately as possible. Gram's stain. $\times 2050$.

M, N, indented heart muscle nucleus.

N, nucleus of body.

V, vacuoles in body.



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PLATE 148

FIG. 2. Fragment of a body photographed at slightly different levels. The constriction of the nucleus and two nucleoli is shown. Gram's stain. $\times 2100$.

A, indented heart muscle nucleus.

B, red blood cell that has floated into the space about the body.

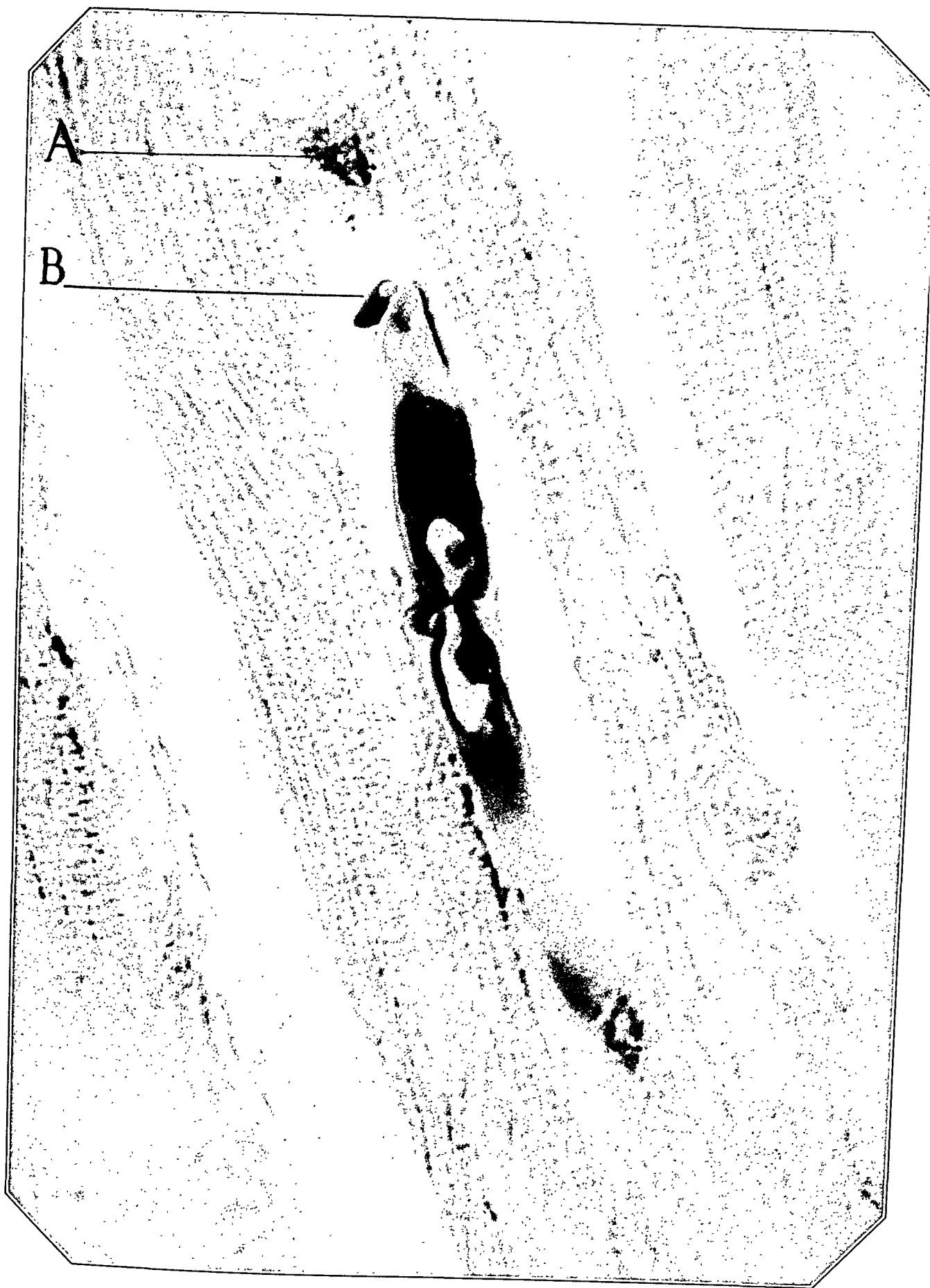


PLATE 149

FIG. 3. Fragments of a body photographed at slightly different levels. The third nucleolus is shown in this figure. Gram's stain. $\times 2100$.

A, indented heart muscle nucleus.

B, red blood cell that has floated into the space about the body.



PLATE 150

FIG. 4. Fragment of a body. Gram's stain. $\times 2100$.



TUBERCULOSIS OF THE MAJOR BRONCHI *

HERBERT S. REICHLE, M.D., AND THOMAS T. FROST, M.D.

(From the Department of Pathology of the Cleveland City Hospital and Western Reserve University, Cleveland, Ohio.)

Little attention has been given in the past to tuberculosis of the major bronchi.¹ Ranke's discussion, though valuable, is cursory and his remarks are scattered among his studies on the classification of tuberculous disease.² Tuberculosis of the major bronchi is, however, of more than descriptive interest since it not infrequently leads to bronchiectasis; the rigidity and size of the diseased bronchi may impede collapse of the lung and interfere with the natural healing of cavities. Furthermore, the studies herein described reveal that the pathogenesis of tuberculosis of the major bronchi has unique features not found in other organs.

In this article the term "major bronchi" refers to air passages that possess mucosa, submucosa, muscle, elastica, mucous glands, an encircling wall of cartilage and a definite adventitia. The material for study was obtained from 37 routine unselected cases of pulmonary tuberculosis, mostly chronic phthisis (tertiary isolated pulmonary tuberculosis of Ranke's classification), but also cases of childhood tuberculosis (primary pulmonary tuberculosis with generalization) and of miliary tuberculosis. The lungs were either fixed by injection of formalin into the trachea after removal from the chest, or hardened *in situ* by injection of formalin into the inferior vena cava. Identified blocks were cut, embedded in paraffin in the usual fashion and stained with hematoxylin-eosin.

Gross examination of the bronchi yielded little of importance. No necrosis of the major bronchi was found although a thickening of the mucosa was often present. The gross appearance was deceptive, since an apparently well preserved wall might be the seat of extensive tuberculosis. Diffuse involvement which apparently would preclude a restoration *ad integrum* was found, however, only in phthisis with extensive cavitation or gross bronchiogenic spread, and even here bronchial mucosa which was but a few centimeters removed from the active focus was usually free from histologically specific

* Received for publication April 27, 1934.

signs of tuberculosis. In such cases edema and a slight diffuse infiltration with lymphocytes were the only evidences of disease, an observation that is particularly striking since large quantities of infectious sputum must have passed over this mucosa daily for weeks and perhaps months. In this fact is found one of the principal arguments against the assumption that tuberculosis of the bronchi usually develops by implantation, a hypothesis that has hitherto seemed plausible to some observers because of a specious comparison of bronchus with intestine. Intestine, however, has an absorbing surface whereas the bronchus has not; in fact, the latter is equipped with strong barriers against the invasion of microorganisms by the possession of cilia, mucous glands and peristaltic activity.

The most striking characteristic of tuberculosis of the major bronchi is the tendency for the disease to affect the mucous glands. The earliest manifestations of tuberculous adenitis cannot be distinguished from the simple lymphatic infiltration noted above. Tubercles appear which may be of the dense proliferative type or may consist of a central core of caseation with a wide zone of perifocal reaction. In either case the process is definitely *between* the acini of the gland. The latter are pressed aside, either undergoing atrophy or, owing to obstruction of their ducts, becoming dilated. This is additional evidence that the infection does not proceed from the bronchial lumen by way of the collecting tubules of the mucous glands.

The predilection of tuberculosis for the mucous glands is all the more striking because glands of the same structure and analogous functions elsewhere, *e. g.* salivary glands and pancreas, are not especially prone to show tuberculous disease. Furthermore, the mucous glands themselves have rarely shown any disease in cases of pure acute miliary tuberculosis, although here and there a few scattered tubercles may be found in the foci of lymphatic cells of the submucosa. Tuberculosis of the mucous glands is therefore usually associated with a tuberculous bronchitis and is probably not due to a hematogenous distribution.

An explanation for the rôle which the mucous glands play in tuberculosis of the major bronchi is found in the minute anatomy of these structures. On the basis of publications by Lewis and Stöhr,³ Piersol,⁴ Macklin,⁵ Berry, Brailsford and Daly,⁶ and Miller,^{7,8} it is possible to outline the pertinent data. Cartilage, muscle and elastic

tissue of a major bronchus represent an almost faultless wall interposed between submucosa and a rather loose adventitia. The main groups of mucous glands are found between the cartilage and the muscle. External to the cartilage lobules of these glands are also found lying in the adventitia, hugging the ring of cartilage on one side and in intimate contact with the peribronchial alveoli and lymph nodes on the other. Ducts from these lobules pass through the elastic membrane between the links of the cartilage, pierce the muscle and internal elastic membranes to enter the submucosa and finally reach the lumen of the bronchus. Sometimes this extracartilaginous glandular tissue is but an outpouching of the mucous glands internal to the ring of cartilage. In the adventitia and often close to the extracartilaginous glands are the lymphatics and venous tracts which transport fluid from the periphery of the lung to the hilum. All the highly infectious material carried from a parenchymal focus must in large part pass through these avenues. The lymphatic flow in the bronchus itself is from the submucosa out toward these main pathways; it is therefore centripetal. Veins and arterioles pass through the same clefts as the extracartilaginous glands.

This portion of the mucous glands is a portal of entry in tuberculous bronchitis and the mode of infection may be called "*infection by contiguity*." The source may be a tuberculous pneumonia of the peribronchial alveoli. Such a condition is found in and near cavities where the bronchial wall usually resists destruction much longer than the parenchyma, and the tuberculous process outflanks, as it were, the bronchus. More important, however, are tuberculous lymphangitis and phlebitis of the vessels which pass toward the hilum in immediate proximity to the extracartilaginous glands. In extensive disease of the parenchyma such changes are practically never absent and unless fibrotic induration of the bronchial adventitia with atrophy of the extracartilaginous mucous glands occurs, a spread to the interior of the bronchus is inevitable. Diseased lymph nodes furnish another source. Tuberculous disease which affects the peripheral portions of these nodes is prone to spread to the adventitia of the bronchus and from there to the mucous glands. Severe pneumoconiosis will develop exactly the same condition and it has also been described in tumor metastases.⁹

Implantation tuberculosis of the major bronchi appears to occur usually after massive irruptions of caseous material. It may be seen

in secondary bronchi of a lower lobe which is the seat of a bronchiogenic distribution from a large apical cavity. Apparently the mass of the infectious material and the effect of gravity in the bronchi of lower lobes counteract the protective action of cilia, mucus and bronchial peristalsis. Hence implantation tuberculosis was not seen in the larger bronchi draining the upper lobes, even though the blocks were taken but a few centimeters from a large cavity.

In these cases a different mechanism of infection prevails — *infection by continuity*. Tuberculous granulation tissue and caseation creep by degrees along the submucosa, destroying the mucous glands, elastica and muscle even before the mucosa itself disappears. Cartilage resists the destructive influences for a longer time and may be found in the midst of tuberculous granulation tissue. Its ultimate destruction is apparently consummated by a metaplasia into a fibrillar substance which is arranged tangentially to the central cartilage and in which are found small pyknotic nuclei. In some instances granulation tissue erodes the cartilage and bone formation occurs. In infection by continuity the mucous glands are not selectively chosen for attack; the pathological process infiltrates all tissues. That even under these disadvantageous circumstances the bronchial wall exhibits considerable resistance was shown in several instances by sections of serial blocks cut from main bronchi draining large tertiary apical cavities. The wall of the primary bronchus was well preserved; here and there a few tubercles were found in mucous glands or submucosa, but for the most part nothing but edema and a sparse infiltration with lymphocytes was seen up to the point where the secondary bronchi entered. Here the wall of the main bronchus showed a rather abrupt atrophy; muscle and elastica disappeared, the cartilage dwindled in size and the submucosa became a mass of granulation tissue in which were found islands of atrophic mucous glands. This granulation tissue led directly into the pyogenic membrane of the cavity and it was not possible to determine where the secondary bronchi ended and the cavity began. Even far in advance of the tuberculous process fibrosis of the adventitia and atrophy of the extracartilaginous mucous glands were apparent; thus the bronchus is protected from the effects of contiguous disease. Collapse is, however, impeded and obliteration of the lumen prevented by the dense hyalinized connective tissue which forms in adventitia and submucosa.

SUMMARY AND CONCLUSIONS

Tuberculosis of the major bronchi can be classified according to pathogenesis as (1) infection by implantation, (2) infection by contiguity, and (3) infection by continuity. The first is decidedly less common than the other two, a fact that may be ascribed to the protective influences of cilia, mucus and bronchial peristalsis. Infection by contiguity is prone to occur because of the proximity of the extracartilaginous mucous glands to diseased lymphatics and lymph nodes. Proliferation of the adventitia and atrophy of the mucous glands tend to close this avenue. Infection by continuity occurs secondarily to an implantation tuberculosis in the lower lobes and in the bronchi draining a tuberculous cavity. Major bronchi appear to be especially resistant to tuberculosis; however, when fibrosis has transformed them into rigid tubes and mucosa and submucosa are destroyed and replaced by tuberculous granulation tissue, an open focus of disease is created which is rarely closed either by natural processes of healing or by pneumothorax or thoracoplasty.¹⁰ We therefore suggest that the critical aspect of a tuberculous cavity is not so much its size *per se* or the density of its wall, as its relation to a major bronchus.

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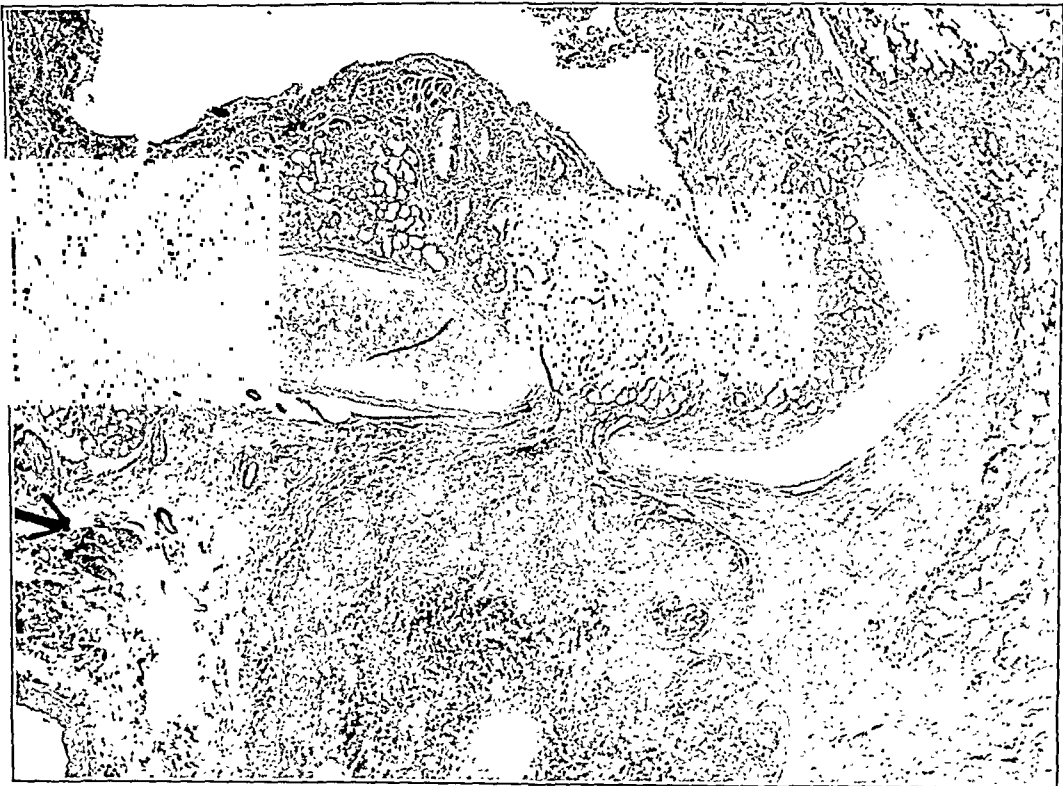
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PLATE 152

- FIG. 3. Longitudinal section of a bronchus showing advanced tuberculous disease by continuity. Note preservation of mucosa far into diseased area. Hematoxylin and eosin stain. $\times 10$.
- FIG. 4. Infection by contiguity. Tuberculous lymphadenitis with invasion of submucosa between clefts of cartilage. Note tuberculous disease of lymph vessels or venules and their proximity to extracartilaginous mucous glands. Hematoxylin and eosin stain. $\times 10$.



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4

SCIENTIFIC PROCEEDINGS OF THE
THIRTY-FOURTH ANNUAL MEETING
OF THE
AMERICAN ASSOCIATION OF PATHOLOGISTS AND
BACTERIOLOGISTS

HELD AT THE BANTING INSTITUTE,
UNIVERSITY OF TORONTO,
TORONTO, ONTARIO,
MARCH 29 AND 30, 1934

THE AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS

ABSTRACT OF BUSINESS SESSION

President AVERY in the Chair

The Secretary presented the nomination of the Council for officers as follows:

<i>President</i>	WILLIAM BOYD
<i>Vice-President</i>	N. CHANDLER FOOT
<i>Treasurer</i>	F. B. MALLORY
<i>Secretary</i>	HOWARD T. KARSNER
<i>Incoming Member of Council</i>	E. B. MCKINLEY
<i>Assistant Secretary</i>	ALAN R. MORITZ

Voted unanimously to elect those nominated.

Voted to elect the following new members:

Arthur L. Amolsch	Olive Gates
A. B. Baker	Philip H. Greey
Eustace L. Benjamin	Arthur W. Ham
Louis F. Bishop, Jr.	Theodore R. Helmbold
Maurice Brodie	Louis A. Julianelle
Jesse L. Carr	Harold B. Kenton
Albert E. Casey	Morton McCutcheon
W. H. Chase	Herbert S. Reichle
Mortimer Cohen	Paul D. Rosahn
William D. Collier	Nathan A. Womack
Martin H. Dawson	David A. Wood
Edgar C. Fielden	Angus Wright
Leroy D. Fothergill	

It was also voted to reinstate Dr. Harold E. MacMahon.

Voted to accept with regret the resignations of Drs. William Bloom, J. W. Churchman, H. R. Dean, E. C. Dickson, W. W. Ford, W. C. Quinby, O. H. Schultze, J. D. Weis, Benjamin White, Anna W. Williams and L. B. Wilson.

Voted to record with deep regret the deaths of Drs. L. H. Braafladt, F. P. Gorham, A. L. Grover, P. E. McNabb and W. Ophüls.

The Secretary reported that the Symposium for 1935 would be held in collaboration with the American Association of Immunologists on the topic of Virus Diseases, and that Dr. T. M. Rivers had been selected as referee.

The Secretary announced that the next annual meeting of this Association will be held at Cornell University Medical College, New York City, April 18 and 19, 1935.

The Scientific program followed.

AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS

PRELIMINARY OBSERVATIONS ON THE CHEMICAL RELATIONSHIP OF ANTIBODIES
TO THEIR ANTIGENS. H. J. Perkin (by invitation), Toronto, Canada.

Abstract. The purpose of this investigation was to study the chemical relationship between the antigen, antibody and the precipitin compound formed by their combination. Iodo-albumin was chosen as a suitable antigen since it produces a strong antiserum and is capable of quantitative analysis in the presence of other proteins. A very pure form of iodo-albumin containing 9.55 per cent of iodine was prepared. It was demonstrated that 40 mg. of this protein injected intravenously into rabbits induced maximum antibody production.

The blood iodine of rabbits immunized against iodo-albumin was found to be increased above the normal and control group. The greater part of this iodine was organically combined and present in the serum, although there was no direct relation between the increased blood iodine and the degree of immunity.

Addition of antigen to immune serum did not show precipitation of all the added antigen. However, the supernatant serum still showed precipitation on further addition of either antigen or antibody. A point of optimum precipitation with relation to the amount of antigen and certain conditions of precipitation was determined. Under these optimum conditions iodine analysis of precipitated and "non-precipitated fractions" showed that less than 50 per cent of the antigen was precipitated. Dissociation of the insoluble precipitate to form a soluble fraction was demonstrated when the two fractions were allowed to remain in contact.

The original purpose of determining whether the increased iodine in the immune serum was associated with the antibody could not be accomplished by the methods used, since the iodine of the serum represented only 5 per cent of the antigen iodine necessary to precipitate it, and the experimental error in iodine estimations of such small amounts was 6 per cent.

Recovery of 3.4 mg. of precipitin with an iodine content of 0.7 per cent suggested that if a chemical union takes place it might be in the proportion of 1 part of antigen to 13 of antibody, assuming that no other serum proteins were carried down in the precipitate. Analysis, after washing the precipitate, suggested that perhaps the antibody may be precipitated by the antigen without the latter necessarily forming any fixed union. However, the experimental evidence to date is inadequate to justify the drawing of any definite conclusions concerning the biochemical relationship between the antibody molecule and the corresponding antigen.

Discussion

(Dr. W. L. Holman, Toronto.) Realizing the large amount of antigen found necessary in producing precipitation with the sera of rabbits after prolonged periods of immunization, we are planning to use sera of animals at an earlier stage in the process when, according to Manwaring, the precipitin complexes

are apparently larger in size but fewer in number. If sufficient precipitate for the iodine determinations can thus be obtained we may approach close to proportions of antigen and antibody, which may help in solving our original problem by the analysis of the possible iodine content of the antibody complex.

THE PREPARATION, PROPERTIES AND APPLICATIONS OF LYOPHILE SERUM PROTEINS AND COMPLEMENT. Stuart Mudd and (by invitation) John Reichel, Earl W. Flosdorf and Harry Eagle, Philadelphia, Pa.

Abstract. Certain applications of familiar conditions, namely the use of low temperature and absence of moisture and oxygen, for preservation of labile substances are here presented.*

A practical method and apparatus is described by means of which water may be removed under a high vacuum (0.005-0.05 mm. Hg.) from biological products in a frozen state. Quantities as large as 100 liters (e.g., the serum from several horses), or as small as 0.1 ml. may be processed. The material is frozen by immersion of its containing vessel in a mixture of acetone and solid CO₂ at -70 to -80° C and immediately placed under high vacuum. Evaporation then proceeds at a rate sufficient to maintain the frozen state. The vapor is condensed in a suitable trap immersed in a cold bath of acetone-solid CO₂ mixture.

The processing of many liters in bulk is of value for purposes of storage and concentration. The processing of small amounts in individual containers is of value in that it makes possible the preservation and distribution of labile materials in any quantities. The apparatus described is capable of handling simultaneously any number of containers, from one to several hundred, each holding from 0.1 ml. to 500 ml. Upon completion of the process the containers are sealed off by fusing the glass exhaust tube with a torch, thus providing for storage in vacuum in individual sealed containers. All operations involved in the entire process are easily carried out with sterile technique.

Quantitative tests on complement and antisera kept at various temperatures indicate that the period over which they retain their original potency is greatly prolonged by this method of preservation. Thus, complement so processed has shown no demonstrable deterioration after 4 months at room and icebox temperatures, and diphtheria antitoxin and antipneumococcus sera have maintained their protective titer for the maximum period tested, namely 2 months, at 50° C. Tests are of course being continued for longer periods. The method makes available to the serological laboratory a pooled and stable complement, eliminating the necessity of daily bleeding and assuring a uniform product. It affords an improved means of preserving antitoxic and antibacterial sera, and a convenient means for their storage and distribution.

Finally, the process is making possible a wider exploration of the possibilities in the prophylactic and therapeutic use of human serum. A collection of human convalescent and adult sera preserved by this method has already been begun in Philadelphia. Clinical experience with these sera in measles prophylaxis has been favorable.

* Among those whose previous use of these conditions for preserving such substance we would acknowledge are Professor W. J. Elser of Cornell, Drs. L. F. Shackell, D. L. Harris, L. A. Rogers, Homer F. Swift, F. M. Huntoon and James Craigie.

Discussion

(Dr. E. G. D. Murray, Montreal.) I should like to ask Dr. Mudd whether he weighed very large quantities in doing the estimations of retained potency of this dry complement. I think in most laboratories we have to weigh very small quantities, and due to the error in weighing most of us do not get a straight line, as shown by Dr. Mudd. I should like to ask him what difficulties in the application of this work arise from the error due to weighing dry material.

(Dr. Lloyd Felton, Boston.) A question comes to my mind in a comparison of the dried and liquid complement. I could not tell from the chart whether or not Dr. Mudd meant that he added water to the dried material and kept it in that way. Of course most people interested in immune serum have dried it for years. I remember as a medical student I dried complement on filter paper and kept it for weeks at a time. The interesting thing in this paper is that there has been a method developed which can be made of commercial application in furnishing dried material, but I do not see how in the comparison between the dried and liquid materials there can be any advantage of the former over the latter. May I ask Dr. Mudd why the reconstructed complement is superior in keeping qualities in the icebox to the fresh complement bled and kept in the icebox?

(Dr. Mudd, closing.) In the tests shown complement was not weighed in the dried form. We redissolved the processed complement by making up to original volume with distilled water. Pooled complement preserved in the "lyophile" form at room temperature, and redissolved, has been found by Dr. Eagle to be identical in activity and keeping qualities with freshly pooled complement, within the limits of Dr. Eagle's method of titration (± 10 per cent).

The method described does not endow sera with any activities they do not originally possess; it does allow for the preservation of sera, apparently for an indefinite time. Two hospitals which have used lyophile pooled complement for routine Wassermann tests have found it both economical and convenient; a complement of known and constant activity is thus made available for use at any time.

In the case of human adult or convalescent serum the method is of service in that it permits sera to be collected when available and utilized when needed.

There has been established at the Children's Hospital, under the direction of Dr. Joseph Stokes, Jr., what we are calling the Philadelphia Serum Exchange. Pooled adult human serum and convalescent serum from diseases in which passive immunization is practicable are collected from Wassermann-negative persons, passed through a bacterial filter as an extra precaution, and processed with sterile precautions. The sera are redissolved by addition of distilled water and injected intramuscularly. The sera so preserved have been used successfully for measles prophylaxis in the children's wards of five Philadelphia hospitals and in a considerable number of cases in private practice. Smaller numbers have been protected against chicken-pox. Pooled adult serum is also being injected, practically as a routine procedure, into patients entering the Children's Hospital, as a prophylactic against possible exposure to contagious disease before admission. An additional advantage of preserving serum in the "lyophile" form is that it permits concentration of the serum.

EXPERIMENTAL VACCINATION WITH B.C.G. B. J. Clawson, Minneapolis, Minn.

Abstract. Rabbits were vaccinated with 1 mg. of B.C.G. at 4 weekly intervals by the four following methods: (1) subcutaneously with living organisms; (2) intravenously with living organisms; (3) subcutaneously with heat-killed organisms; and (4) intravenously with heat-killed organisms. The results were studied with respect to safety and resistance.

Safety: The degree of safety was estimated from the presence or absence of tuberculous lesions in the lungs, liver, spleen and kidneys in from 3 to 16 weeks after the last injection of the vaccine, and from the presence or absence of allergy, as indicated by the Mantoux test. The lesions observed were microscopic and in no case showed any evidence of becoming progressive.

Lesions were found only in animals vaccinated intravenously with living B.C.G. and only in the allergic animals of this group. Allergy was present in a high percentage in the animals vaccinated by all the methods, except in those vaccinated intravenously with heat-killed organisms.

From the standpoint of lesions and allergy the method of administering the B.C.G. vaccine as heat-killed organisms intravenously carries with it the greatest degree of safety.

Resistance: Three weeks after the last injection of the vaccine another group of animals was injected intravenously with 0.01 mg. of a virulent bovine strain.

It was observed from the greater length of life in the vaccinated than in the non-vaccinated animals, from noting the less amount of involvement or absence of infection in the vaccinated animals, and from the absence of any resistance in the animals vaccinated with the timothy grass bacillus, that vaccination with B.C.G., living or heat-killed, gave a degree of specific resistance. While resistance was present in all four groups, it seemed to be greatest in the groups vaccinated subcutaneously with living B.C.G., and intravenously with heat-killed B.C.G.

The experiments seemed to show that efficient vaccination with B.C.G. may be safe and that, while allergy may be associated with resistance, as great or a greater degree of resistance against a tuberculous infection may be developed without a measurable amount of allergy. Vaccinating intravenously with heat-killed organisms seemed to be the best method to develop resistance with safety.

Discussion

(Dr. E. G. D. Murray, Montreal.) I think that we still have to be very cautious in drawing conclusions from data of this kind in applying this immunization to humans because, in the first place, relatively few animals were used in the experiments and, in the second place, it is dangerous to argue from animals to man. It has been shown by Stanley Griffith, who most of you know is a very cautious worker and who I know is rather interested in B.C.G. immunization and has had great hope of it, that although he can immunize calves, guinea pigs and rabbits, he has failed completely to immunize monkeys against tuberculosis, either bovine or human. On that account I think, in spite of the evidence of a lot of this kind of work which is accumulating, we still have to be very cautious before applying this method to human beings, even leaving out of consideration the possibility and probability of dissociation of this attenuated strain into a virulent phase, as several reliable workers have demonstrated.

(Dr. Esmond R. Long, Philadelphia.) The results presented by Dr. Clawson are convincing and are quite in accord with other experience on immunization with attenuated forms of living tubercle bacilli or with dead bacilli. We have always been able to secure some immunity, although not so good, with the old Saranac Lake strain, R I. I agree with Dr. Murray, however, that we should be very cautious in transferring these results to human beings. The situation as regards human tuberculosis is so different from that of experimental tuberculosis that we have to take special precautions in the interpretation of experimental results.

I should like to ask Dr. Clawson if he noticed any difference in the anatomical character of the tuberculosis in the controls and in those that had received the vaccination. We all know that we have at least two distinct kinds of human tuberculosis, the tuberculosis of childhood and the adult type. The point has frequently been made that we cannot expect B.C.G. to be very effective in the prevention of adult tuberculosis when we realize that adult tuberculosis develops in the presence of an old childhood tuberculosis. If a childhood invasion which is sufficient to make a calcified nodule in the lung is not sufficient to prevent the later development of tuberculosis, how can we expect that B.C.G. will do it? On the other hand, it seems to me that this type of immunization may be worth while in protecting against these progressive childhood infections which are responsible for a certain amount of our tuberculosis mortality.

(Dr. Clawson, closing.) I have only reported data on rabbits. The results seem to be as good, if not better, in protecting guinea pigs against the human strain.

In regard to the differences in the character of the lesions in vaccinated and unvaccinated animals I do not want to speak definitely, but I think there was more necrosis and a great many more organisms in the lesions in the non-vaccinated animals. In many lesions in the vaccinated animals it was impossible to find organisms at all. In most of the lesions in the non-vaccinated animals the organisms were distinct and could be seen by holding the tissue up to the naked eye.

The question which Dr. Long raised about the childhood tuberculosis having a protective effect against subsequent infection raises the question of just what degree of immunity such children do have as a result of just one lesion. I attempted to measure that to some extent by experiments. I made a series of animals allergic by giving them a large injection of B.C.G. subcutaneously, and then measured the concentration of the antibody by determining the complement fixation and agglutination titers. I then took another series of animals and vaccinated them intravenously with heat-killed organisms, and the titers were decidedly greater, which suggested that by vaccinating those that are allergic an increased immunity may be induced.

THE PATHOGENESIS OF ACUTE ANTERIOR POLIOMYELITIS. Maurice Brodie (by invitation), New York City.

Abstract. In order to determine whether or not the nasal cavities constitute the portal of entry for the virus of poliomyelitis and also to determine whether or not the virus travels along the olfactory nerves a bilateral section and partial removal of the bulb and tract was carried out on a series of *Macacus rhesus* monkeys by means of a transfrontal approach prior to intranasal administration of virus. The animals with cut olfactory nerves resisted intranasal inoculations

of the virus, in one experiment as many as 12, whereas the controls became paralyzed following a short incubation period after a single inoculation.

The nasal mucosa is innervated not only by the olfactory nerves, but also by branches of the fifth and seventh cranial nerves. In addition, virus can percolate from the nasopharynx to the tonsils with its intact nerve supply. Yet, upon cutting the olfactory tract no infection occurred, indicating clearly that the first cranial nerve is the only one of the nasopharynx that can transmit the virus of poliomyelitis from the nasopharynx to the central nervous system. Over a period of 3 weeks 2 experimental animals received 12 intranasal inoculations, of which considerable must have dribbled into the gastro-intestinal tract. This observation discounts the gastro-intestinal tract as the portal of entry.

When animals with severed spinal cord but closed dura and an intact hemogenous system and flow of spinal fluid were injected into the upper or lower segment the virus failed to pass the gap in the cord, showing that the virus spreads along nerve tracts and not by the spinal fluid or blood. Inasmuch as the perineural lymph spaces of the olfactory nerves continue into the subarachnoid space and the nerve fibers into the rhinencephalon the virus must travel along the nerve fibers. A correlation between the infectivity of the central nervous system of monkeys, at the acute stage of the disease, with the amount of nerve cell destruction and also the loss of infectivity with removal of neurones, indicates that most of the virus is lodged in the neurones.

Experimental poliomyelitis, then, is entirely neurotropic; the virus travels along the olfactory nerve fibers to the central nervous system where it is propagated along the nerve tracts. Since only the olfactory nerve of the nasal cavity can carry the virus and because no infection was obtained when large amounts of virus reached the gastro-intestinal tract the portal of entry must be the nasal cavities.

ST. LOUIS ENCEPHALITIS — EXPERIMENTS ON PATHOGENESIS AND IMMUNITY. Leslie T. Webster and (by invitation) George L. Fite, New York City.

Abstract. Susceptible mice given an intranasal instillation of the virus of St. Louis encephalitis develop, after a 5 to 6 day incubation period, an encephalitis that terminates fatally in 7 to 10 days. Their bloods and spleens rarely contain detectable virus at any stage of the infection. Their brains, however, show virus 2 days after infection and constantly thereafter, in amounts increasing to a maximum by the 6th day, when signs of disease first appear. Lesions in the anterior region of the olfactory lobes are demonstrable on the 3rd day after infection. On the 4th day the lesions extend to the lobus piriformis, and on the 5th day to the cornu Ammonis.

Mice given an intraperitoneal or subcutaneous injection of 1000 lethal doses of the virus do not develop encephalitis but remain well. The injected virus is detectable in the blood, however, in considerable quantity within 10 minutes, but disappears after a few hours, depending on the size of the infecting dose. In the spleen virus persists for at least 7 days.

These mice injected intraperitoneally or subcutaneously become immune, within 7 days, to 1000 intranasal or 1,000,000 intracerebral lethal doses. Indeed, a single subcutaneous or intraperitoneal dose as small as 0.000001 gm. brings about this high grade immunity, persisting unchanged for 5 weeks and doubtless much longer.

SEROLOGICAL RELATIONSHIP OF ST. LOUIS AND JAPANESE ENCEPHALITIS. Leslie T. Webster and (by invitation) George L. Fite, New York City.

Abstract. The recent outbreak of encephalitis in and about St. Louis, Mo., resembles epidemiologically and clinically the encephalitis in Japan designated Type B. A further comparison of the two diseases has now been made by means of a serological test based on the fact that the virus of the St. Louis disease mixed with serum from convalescents and injected intracerebrally into mice is specifically neutralized. Fifteen sera from cases in Japan have been tested — three from persons with encephalitis in August, 1924, and twelve from persons with encephalitis in August, 1933. None of the sera showed any protective action against the St. Louis virus.

Discussion

(Dr. Ralph D. Lillie, Washington.) I should like to ask what proportion of the sera from the St. Louis outbreak would protect against the virus which came from the same outbreak.

(Dr. Howard T. Karsner, Cleveland.) This work has justifiably attracted widespread attention. The large volume of material presented in a short time has left me uncertain as to whether or not Dr. Webster and Dr. Fite have established the identity of these viruses as separate and distinct from others, for example, that of herpes.

(Dr. T. M. Rivers, New York City.) I am certain a few words can clarify the situation for Dr. Karsner. In the first place it may be rather difficult for those not familiar with viruses to understand how two virus diseases of the central nervous system may present the same clinical, epidemiological and pathological picture, and yet be quite different serologically. The St. Louis encephalitis and the Japanese summer encephalitis are similar, if not identical, clinically and pathologically but the active agents involved are immunologically different. The brain responds to injury in a limited number of ways. Consequently, different etiological agents can produce the same clinical and pathological picture. There is no doubt of the fact that the sera which came from Japan do not neutralize the virus recovered from the St. Louis epidemic. Among the diseases of lower animals one finds good examples of the state of affairs about which I have just spoken. In horses, for instance, an encephalomyelitis occurs in the East and in the West. The disease in the two localities is similar clinically but different immunologically. The same is true of foot-and-mouth disease: different strains of virus produce the same clinical and pathological picture, but differ immunologically. Furthermore, in vesicular stomatitis there are two strains of virus which cause the same clinical picture but differ immunologically. One does not need to illustrate the point in question in more detail. It is not surprising, therefore, that in human beings there are virus diseases which resemble each other very closely and yet are caused by active agents immunologically different.

(Dr. Lloyd Felton, Boston.) Dr. Rivers has answered the question I was going to ask, but I should add this comment — that occasionally we need a little mental stimulation when it comes to medical research. Some people do; others don't, but I do. When Dr. Webster came to Boston and gave his paper I was sure if Paul de Kruif had been present he would have made a picture of it. To me it was a beautiful piece of work done in such a short time and in such

conclusive fashion. Here is the picture of an epidemic in the West and the doctors running around and trying to do everything they can to help. Dr. Webster goes out and with the greatest spirit of coöperation with the Westerners he detects the virus and injects it into mice. He gets it out and follows it, as the picture shows you. It was a lot of work, requiring a lot of patience. To me it is such a pretty thing that although I am a pessimistic optimist it does give a worthwhile slant on medical research, and really doing things that can be done in a clear-cut fashion. I think Dr. Webster and the people who coöperated with him deserve a great deal of commendation for the very excellent way they have conducted this research.

(Dr. Maurice Brodie, New York City.) Regarding what Dr. Fite has brought out concerning the Japanese epidemic being different from the St. Louis epidemic, there is a possibility of the same thing in polio, as pointed out by Australian workers. In studying human strains we have found immunological differences between two recently isolated strains and the monkey passage virus which we are studying. Whether there is no relation whatever between them, or whether it is merely quantitative we cannot as yet say, but we know there is a difference. We have done some work with the encephalitis virus, and the first question was whether it was herpes, and I think Dr. Fite pointed that out pretty well, but we have done other work on it. The fact that you cannot infect guinea pigs speaks pretty definitely against its being herpes. We took a series of forty normal sera, and whereas over 50 per cent of normal individuals show neutralizing substances to the herpes virus no one showed neutralizing substances for this virus.

(Dr. Fite, closing.) In regard to the percentage of the St. Louis cases that neutralized the virus I am sorry I have not the figures here, but over 90 per cent of those sera that were taken more than 10 days after the onset of the disease did neutralize it. Those taken earlier for the most part did not.

Dr. Brodie has answered pretty well the argument against this virus being the herpes virus. It does not infect guinea pigs or rabbits. Inoculation of the eye, skin or brain of a rabbit produces no fever, no restlessness, no sign of herpes whatever. Herpes sera fail to neutralize the virus in any degree. There are many of the same sort of reasons for believing it is not identical with the virus of equine encephalomyelitis or vesicular stomatitis. We ourselves, who happen to be familiar with the virus of louping-ill, know there is no cross-neutralization between the sera of either one with the other virus.

THE GROWTH OF CAPSULAR SUBSTANCES INDEPENDENTLY FROM THE BACTERIA. Louis Dienes, Boston, Mass.

Abstract. Certain bacterium strains produce in excessive amounts capsular substances and their growth is the cause of such phenomena as the ropiness of beer, mucoid fermentation of dough, and so on. We studied subtilis strains showing this property and observed that on saccharose agar plates the mucoid extrabacterial material grows out from the colonies to a considerable distance (up to 20 mm.) on the surface of the agar, forming a halo around the colonies. The halo contains no bacteria and transplants of it on the usual media remain sterile. On saccharose-containing media the transplants have been cultivated in 4 generations as a transparent material in which bacteria were not reproduced. In preparations stained with flagellar staining methods the capsules of the bacteria appear as a dense, hair-like growth of fine filaments. The capsules

of pneumococcus and of other different bacteria appear to be similar. The extrabacterial substance in the subtilis colonies consists mostly of the above mentioned fine filaments. There are many degenerating bacteria in the culture which often show polar staining, and many polar bodies are lying free among the bacteria. In preparations from the halo, stained with flagellar staining methods, we found different morphological elements: long, fine filaments which represent probably the element on which the expanding of the halo depends; short, very fine filaments connected with granules; small, round or oval, spore-like bodies which sometimes form with the filaments asteroid figures; and broken-off parts of thicker filaments which sometimes grow out from the spore-like bodies. The morphological elements of the halo are different from the elements of any known bacterial culture but present close similarity to the elements found in the cultures of the viruses of *pleuropneumonia bovis* and agalactia. The growth phenomena and the definite morphological structure of the halo furnish convincing evidence that the production of the halo represents the growth of living elements different from the usual bacterial forms. The structures which are regarded as a capsule around the bacterium may assume a quite different biological significance. As every spore from a heated spore emulsion reproduces the halo it is probable that the elements of the halo are derived from the bacteria.

Discussion

(Dr. Marcus W. Lyon, South Bend.) I should like to ask Dr. Dienes how he stains these structures. They look like flagella in some instances and in others are quite different.

(Dr. Dienes, closing.) The filamentous structures in the culture and the capsule of the pneumococcus are stained best by Loeffler's method, while the elements in the halo are stained best by Zettnow's method. These filamentous structures are certainly different from flagella, because they can be stained beside each other in the same preparations.

THE PROTEASES AND ANTI-PROTEASES OF PLEURAL EXUDATES. Charles Weiss and (by invitation) E. J. Czarnetzky, San Francisco, Cal.

Abstract. Not received.

TISSUE REACTIONS IN IMMUNITY: TISSUE-ANTIGEN REACTIONS IN PROTEIN-IMMUNIZED RABBITS. Reuben L. Kahn and (by invitation) Elizabeth L. McDermott, Ann Arbor, Mich.

Abstract. When specific protein is injected into some fixed tissue, such as the skin or muscle, of a protein-immunized rabbit the protein is localized at the site of injection and an inflammatory response is soon noted. It is believed that the tissue combines with the protein, thereby anchoring it and preventing it from diffusing throughout the body, and that the inflammatory response tends to wall off the injected area and destroy the protein. With a standard method of immunization a given tissue possesses a combining capacity for a certain quantity of protein only. When this quantity is exceeded uncombined protein finds its way to the other tissues of the body. Also, different tissues of protein-immunized rabbits possess different capacities for combining with the protein, the cutaneous and peritoneal tissues possessing combining capacities approximately ten times as great as those of *in vivo* plasma and of muscle and brain

tissue. If a protein-immunized rabbit is given an intravenous injection of the specific protein and soon after the same protein is injected into the skin, no local inflammatory response will be noted. As a result of the intravenous injection the animal is disimmunized (desensitized) temporarily.* The term disimmunization is preferred to desensitization, which is used to express clinical conditions unrelated to these experiments. By disimmunity is meant the reverse of the immune state. Only an immune animal can be disimmunized, hence disimmunity is not synonymous with susceptibility or non-immunity. The tissues in the disimmunized state perhaps become "saturated" with the protein and thereby lose their capacity to combine with additional protein.

These quantitative aspects of the antigen-combining capacities of the tissues of protein-immunized rabbits are indicated by a method devised in this laboratory.† The method utilizes horse serum as the immunizing reagent and modified horse serum in the form of antitoxin with its specific toxin as the reagents for measuring the tissue changes as a result of the immunization with horse serum. Thus, if into the skin of a horse serum-immunized rabbit are injected 50 M.L.D. of diphtheria toxin, and simultaneously 750 units of horse serum antitoxin are injected subcutaneously in another area, the rabbit will succumb, indicating that the subcutaneous tissue combined with the antitoxin, thus permitting the toxin which diffused through the body to exert its lethal action unhampered. If 1000 units of antitoxin are injected the rabbit will survive; some uncombined antitoxin presumably escapes from the area of injection and neutralizes the toxin *in vivo*. When a horse serum-immunized rabbit is injected intravenously with a considerable amount of horse serum the rabbit becomes temporarily disimmunized; the skin loses its capacity to combine with antitoxin and 20 units of this reagent will be found sufficient to save this animal from 50 M.L.D. of toxin, similar to the amount of antitoxin required to save normal rabbits.

It is believed that these findings have a bearing on immunity to bacteria, emphasizing particularly the quantitative nature of the immune state of the tissues. The skin, for example, may have a reacting or combining capacity for staphylococci of certain virulence, sufficient to prevent these organisms from permeating into the deeper tissues of the body. This immune manifestation apparently is broken down if the quantity of organisms exceeds the limit of the combining capacity of the skin. The fact too that the skin possesses this combining capacity to a greater extent than muscle or brain tissue would indicate that the property of the skin to prevent the spread of these organisms into other tissues of the body is greater than that of muscle or brain tissue. In a condition where the organisms circulate in the blood stream in considerable numbers we are undoubtedly dealing with a phase of disimmunity. Whereas in the immune state the host is able to keep the infecting organisms localized, in the disimmune state the organisms tend to become widespread throughout the body. The host has the upper hand over the organisms in the immune state, while the organisms have the upper hand in the disimmune state. It should be added that under the conditions of the experiments described the disimmune state is rapidly followed by an immune state usually of a higher level than before disimmunization, indicating that the disimmune state serves as a stimulus to the animal to build up its immunity.

* Kahn, R. L., *J. Bact.*, 1934, 27, 92.

† Kahn, R. L., *Science*, 1934, 79, 172.

TISSUE REACTIONS IN IMMUNITY: TISSUE-ANTIGEN REACTIONS DURING PERIOD OF INCUBATION. Reuben L. Kahn, Ann Arbor, Mich.

Abstract. The period of incubation in these experiments refers to the interval between the time of injecting a protein into a rabbit and the development of the capacity of the skin specifically to react to a second injection of the protein. Little is known regarding the immunological changes in the body tissues of the rabbit during this period of incubation, which is believed to extend for about a week or more. By means of the antitoxin method utilized in the studies presented in the preceding paper it was possible to establish that immunological changes begin to take place in the skin of the rabbit as early as 48 hours after an injection of protein. If a rabbit is given an immunizing injection of horse serum and 2 days later is injected with 50 M.L.D. of diphtheria toxin intracutaneously, and simultaneously in another area of the skin with 20 units or in some cases with 25 units of horse serum antitoxin, the animal will succumb from the toxin. Normal rabbits succumb from 50 M.L.D. toxin when only 15 units or less of antitoxin are injected into the skin, but are saved by 20 units. It is believed that the skin of the 2 day previously injected rabbits develops as a result of the injection a slight capacity to combine with antitoxin, which is essentially specific antigen. Hence, 20 or 25 units of antitoxin are not sufficient to save them from toxin death.

It was further observed that many factors play important rôles in the determining whether tissue changes will take place during this short incubation period or not. Preliminary experiments indicate that the quantity of horse serum injected and whether it is undiluted or diluted with salt solution; the route of injection; the age of the rabbit, especially whether it is in a growing or in a mature state; also the non-specific immune state of the rabbit — all these and undoubtedly other factors affect tissue changes during this period. A relatively large dose of horse serum, injected intracutaneously or subcutaneously, may prolong the incubation period, whereas a small dose of the protein solution is likely to shorten the incubation period and thus hasten the appearance of detectable immune changes in the skin. When the horse serum is injected intravenously, thus far no detectable skin changes have been observed 2 days after the injection. Mature rabbits show more marked tissue changes during the period of incubation than young rabbits. Non-specific immunity also tends to hasten tissue changes. Beginning with 3 days after an immunizing injection of horse serum specific tissue changes show a gradual increase. No attempt has thus far been made to determine tissue changes in less than 48 hours after the injection.

It would appear from these studies that the tissues of an animal during the incubation period following the entrance of bacteria into the body undergo important immunological changes. The extent of these changes may be a determining factor as to whether or not the organisms will be prevented from establishing an infection. Indications are also that the smaller the quantity of organisms gaining entrance the more rapid is the immune tissue response, while with a larger quantity of organisms the oncoming of immunity is likely to be delayed. The general belief that small doses of infecting material tend to immunize, while large doses tend to bring about infection, appears to be substantiated by these studies with protein. That the tissues of mature animals possess greater immune capacity than those of young animals is also of interest. It is hoped that extensive studies of these factors governing tissue im-

munity during the incubation period will throw light on the immune mechanisms of the body tissues during this period and will open the way to special means of stimulating the immune response of the tissues during this same period in infection.

Discussion

(Dr. Stuart Mudd, Philadelphia.) I have been impressed with the recent papers of Dr. Arnold Rich in the field of allergy and immunity. It seems to me that he has advanced the subject importantly by showing that allergy and immunity can be separated. I take it from Dr. Clawson's paper this morning that he also has been able to distinguish and separate immunity and allergy in tuberculosis, the field in which these two conditions have most often been regarded as perhaps inseparably associated. If I understand Dr. Kahn correctly he is introducing the term "disimmunization" and defining it in terms of removal of allergy. It seems to me that introduction of a new term into an already confused terminology is always a doubtful step; in particular the question arises in my mind as to whether Dr. Kahn's term "disimmunization" serves to clarify or merely further to confuse this subject.

(Dr. Louis Dienes, Boston.) From the interesting observations of Dr. Kahn I was mostly interested in the early appearance of specific response. I have studied, together with Dr. Tracy B. Mallory, the first appearance of hypersensitiveness and we found that either in rabbits, guinea pigs or in man it is possible to obtain definite skin reactions on the 4th day after the immunizing injection. We found, furthermore, that the early manifestations of the specific response are qualitatively different from the later manifestations. The early skin reactions do not start to develop with an exudation like the later reactions, and infiltration with mononuclear cells is the most pronounced feature of them. This type of reaction is present on the 4th, 5th and 6th days after treatment; later — and always in passively sensitized animals — the skin test produces a different type of reaction in which exudation plays the most important part. So the mechanism of the specific reactions which the antigen produces at the site where it comes in contact with the tissues is markedly different in the early and later stages of immunization. We could not obtain direct evidence concerning the rôle which the early phase of the specific response plays in the healing of disease, but it seems probable that this rôle is important. The late development of antibodies in disease and the observation that the introduction of antibodies, except in the first few days of disease, exert hardly any influence on it show that the healing cannot be explained simply by the production of antibodies. Such observations as the development of bone lesions or orchitis after the healing of typhoid fever point to the fact that the antibodies are not the only factor in the healing process and the local tissue resistance at the site of the lesions plays an important rôle in it. It is not impossible that the early phase of the specific response, which is purely a tissue response, is more important for the healing than the production of circulating antibodies. The study of the early phase of specific response might give us important new information and it seems to me that Dr. Kahn has indicated a new and a very fine method for this study.

(Dr. E. G. D. Murray, Montreal.) I think the question of the difference between allergy and immunity might lead to an interminable discussion. However, it seems more profitable at the moment to regard allergy as just a stage in the

production of immunity. This work of Dr. Kahn's has a very direct application. The astonishing thing to me is that the peritoneal route in the immunized animal should hold up the antigen to such an extent, because one would have expected on what has been done before that the absorption of antibody introduced by the intraperitoneal route would be only second to the intravenous route. The application of this work is in the administration of immune serum. I have been privileged, very much privileged, by the courtesy of the Connaught Laboratory, to use staphylococcus antitoxin therapeutically during the past year, and it has been very surprising in patients who have some degree of, let us say, sensitiveness to horse serum, what an astonishing amount of that antitoxin has to be administered by the intramuscular route, compared with patients who have had no history of previous administration of horse serum and who showed no degree of what is called allergy. The intravenous route is certainly the most advantageous route, but the serious reactions which followed the administration of serum in certain cases place the patient in a position of very grave danger. Therefore other routes are desirable, and in the administration by the intramuscular route we have found it necessary to "disimmunize" the patient. I should like to ask Dr. Kahn, mainly for my own guidance, how long the stage of "disimmunization" in these animals lasts, because at times with repeated administrations of antitoxin one has to face that problem.

(Dr. Kahn, closing.) I am thankful to Dr. Mudd for bringing up the question of the relation between allergy and immunity. It is true, as Dr. Murray stated, that if we entered extensively into a discussion of this subject we would never get through. It is important, however, to emphasize our different points of view from time to time so that we may all keep this subject in mind. The question under consideration is in reality whether a specific inflammatory response is immunological or allergic in nature. If immunological the inflammation must be interpreted as being protective; if allergic the inflammation must be interpreted as some condition opposite to protection. Pathologists have long ago defined inflammation as a defensive mechanism. If this definition is to stand it seems to me that we must accept the specific inflammatory response in an immunized animal as immunological in character. Therefore, when, by some special means we rob the tissues of the capacity to produce a specific inflammatory response we produce a condition which is the reverse of the immune state, namely, we disimmunize the animal.

To illustrate — a given amount of horse serum, such as 1 cc., is injected subcutaneously in a rabbit. The horse serum soon diffuses from the area of injection and the greater portion is gradually eliminated through the kidneys. Suppose the same quantity of horse serum is injected subcutaneously in the same rabbit some 2 weeks later. The injected horse serum does not diffuse from the area of injection; it remains localized and an inflammatory response soon becomes visible in that area. The inflammation apparently tends to wall off the injected area and destroy the horse serum by proteolysis. But the skin can be robbed of the capacity to destroy the horse serum locally by means of an inflammatory response. All that is necessary is to inject the rabbit intravenously with horse serum. When this is done it may be that all body cells become saturated with the horse serum. Therefore, when this serum is injected into the skin the cells are incapable of combining further with it and of bringing about an inflammatory response. Following the intravenous injection of the horse serum the rabbit becomes disimmunized for a certain number of hours or for a day or two, depending on the quantity injected. The disimmunized state is

followed by the immune state when the skin will again respond with local inflammation to a cutaneous injection of horse serum. It should be recalled that the term desensitization is employed by clinicians to designate the return of allergic patients to normality. Obviously the horse serum-immunized rabbit which has been injected intravenously with horse serum has not thereby been brought to the normal state.

We are all familiar with the studies of local tissue immunity of Dr. Cannon and his associates in the University of Chicago. If tissue responses are basically allergic it seems difficult to understand how we can have local tissue immunity. I have the highest regard for the work of Dr. Rich and Dr. Clawson, but I regret I cannot agree with their interpretation. Dr. Murray raises an important question. The disimmunized state is of very short duration, an animal apparently making every effort to return to the immune state.

AN EFFECT OF ALTERING THE pH OF THE REACTANTS IN THE PRECIPITIN REACTION. James T. Culbertson (by invitation), New York City.

Abstract. Not received.

THE BACTERIOLOGICAL DIAGNOSIS OF ACTINOMYCOSIS. P. H. Greey (by invitation), Toronto, Canada.

Abstract. The clinical data on 20 cases of actinomycosis which occurred at the Toronto General Hospital in 2 years, representing a four-fold increase in incidence, was presented. Practically all of these cases were diagnosed bacteriologically and the procedure used routinely in making such a diagnosis was described.

BACTERIAL VARIATION IN PNEUMOCOCCUS AND STREPTOCOCCUS HEMOLYTICUS. M. H. Dawson (by invitation), New York City.

Abstract. Studies in variation in pneumococcus have shown that this bacterial species possesses a third and distinct variant form in addition to the currently accepted S and R forms. The characteristics of this third variant form are described in detail and the interrelationships between the three chief variant forms are considered.

Studies in variation in *Streptococcus hemolyticus* have demonstrated that this species also possesses three chief variant forms: (1) M (mucoid), (2) S (smooth), and (3) R (rough). Attention is particularly directed to the mucoid form. A comparison of these three chief variant forms of *Streptococcus hemolyticus* with those of pneumococcus reveals a remarkable parallelism in their salient characteristics.

A review of the subject of variation in a wide variety of bacterial species reveals that the general phenomenon of variation fits into a more or less orderly and uniform pattern. The studies on variation in pneumococcus and *Streptococcus hemolyticus* show that variation phenomena in these species conform to that general pattern.

Reference is made to the desirability of adopting a uniform terminology to describe corresponding variant forms in various bacterial species.

Discussion

(Dr. Charles Weiss, San Francisco.) May I ask what is meant by neopeptone?

(Dr. Dawson.) Neopeptone is a digest of casein which is now available commercially from Difco Laboratories, Detroit. Perhaps Dr. Avery will say a word about it.

(Dr. O. T. Avery, New York City.) The neopeptone referred to is a product, the preparation of which is based on the results of studies carried out in our laboratory by Dr. Dubos, who demonstrated the presence in various commercial peptones of substances which are bacteriostatic in the oxidized form. The removal of these toxic products by chemical methods enhanced the growth-promoting properties of media containing the purified peptones. Dr. Dubos showed further that a peptic digest of casein was devoid of bacteriostatic substances and that the use of this product in culture media facilitated the growth of minute inocula of the more difficultly cultivable microorganisms.

THE PATHOGENICITY OF THE GENUS *BACTEROIDES*. D. C. Beaver, Rochester, Minn.

Abstract. The genus *Bacteroides*, as defined by Castellani and Chalmers, and by Bergey's Manual of Determinative Bacteriology, includes certain obligate anerobic, motile and non-motile rods which do not form endospores. French and German investigators have emphasized the importance of this group of microorganisms, stating that they are a frequent cause of suppurative and gangrenous infections of man. Elsewhere in the literature they have been described only rarely.

Veillon and Zuber in 1898 were apparently the first to describe and isolate anerobic organisms of this type. They obtained several species from cases of appendicitis and other suppurative conditions, to which they gave the names *B. ramosus*, *B. serpens*, *B. fragilis*, *B. furcosus* and *B. fusiformis*. Hallé confirmed this work by isolating similar forms from the vaginal flora and from suppurative conditions about the female genital tract. He introduced a new species and named it *B. funduliformis*. Rist and also Guillemot, Hallé and Rist recovered similar species from pulmonary abscesses and putrid empyema. Cottet described them as the cause of phlegmonous periurethral infections of the male. Harris and Norris found types similar to those described by Veillon and Zuber in association with hepatic suppuration. The literature of the subject has been reviewed recently by Teissier, Reilly, Rivalier and Stefanescu (1931), and by Cohen (1932). Teissier and associates placed particular stress on the occurrence of bacteremia in the course of infection with *Bacteroides funduliformis*. They reported 4 cases. In Case 1 there were liver abscesses with apparently secondary abscesses of the skin and kidney. The illness in Case 2 began with sore throat; purulent pleurisy followed. The onset of illness in Case 3 was also with sore throat; purulent pleurisy with some involvement of the lung supervened. Case 4 also had onset with sore throat; secondary abscesses of the sternoclavicular and right sacro-iliac regions and right groin developed. Cohen studied the bacteriology of abscesses of the lung, and concluded that anerobic organisms of the type under discussion were the usual cause.

Of equal importance are the observations of Distaso, Debono, Teissier and Eggerth and Gagnon, that members of the genus *Bacteroides* are abundantly present in the normal human intestinal flora.

The present study confirmed the work of previous investigators. *Bacteroides funduliformis* and other species were found to be important as etiological agents in (1) infections of the male urinary tract; (2) ulcerating carcinomas of the colon; (3) perirectal abscesses; (4) suppurative appendicitis; (5) pylephlebitis; (6) abscesses of the liver; and (7) abscesses of the lung. In most instances there was demonstrable bacteremia. Infections were characterized by suppuration usually with abscess formation; sometimes there was gangrene. Experimental inoculation of guinea pigs and rabbits proved that the microorganisms were virulent for these animals; experimental abscesses similar to those observed in the human resulted. Infection of man by *Bacteroides funduliformis* and other species may provoke a profound toxic reaction, and in many cases the prognosis is extremely serious.

Discussion

(Dr. E. G. D. Murray, Montreal.) I should like to ask Dr. Beaver if he has any reason to account for the fact that he has found so few of these organisms in the fourth class, the female urogenital tract, considering that he has found such an unusual number of cases of infection in the other situations which he has described. In our experience *Bacteroides* can be isolated from the lochia of practically all women postpartum. We have isolated quite a variety of them, which we have considerable difficulty in placing. Among others we have a pigmented form which produces a dense black pigment and has some properties resembling melanin. Considering the great frequency with which these organisms are present in the human vagina it is surprising that there are not more serious conditions such as Dr. Beaver has described. I should like to know if he can explain that. I agree with him that they need a richer medium than is ordinarily described as being necessary for their growth. Usually they won't grow without blood or serum.

(Dr. Beaver, closing.) In reply to Dr. Murray I would state that the only reason I have not found *Bacteroides* in infections of the female genital tract is that the material which I have studied is routine material, and I happen to have access more to colon material than any other type. Probably if I had the same access to infections of the female genital tract, I would find *Bacteroides* as frequently as Dr. Murray and others have.

INCLUSION BODIES IN THE SALIVARY GLANDS AND LIVER OF MICE AND RATS. Juanita Thompson, Washington, D. C.

Abstract. This study was concerned with the spontaneous occurrence of a number of intracellular abnormalities in the salivary glands and liver of mice and rats. Some were found in relation to a highly fatal spontaneous epizootic in which many of the animals presented gross manifestations closely simulating the so-called cutaneous or generalized type of infectious ectromelia. In a selected group of 25 animals inclusions were only demonstrated satisfactorily in five of the livers. The inclusions occurred in both the cytoplasm and the nucleus. The other intracellular formations seen were found in apparently healthy animals. In the mucous acini of the submaxillary glands in both mice and rats large inclusion-containing cells were found in a considerable number of the animals. These histological structures had many features in common with the inclusions associated with guinea pig submaxillary virus disease. In a considerable number of cases unusual intranuclear changes were also observed

in the acinar cells of the facial portion of the parotid glands of apparently healthy rats and in the hepatic cells of seemingly normal mice. The changes were comparable to the intranuclear inclusions reported by Findlay in the livers of a strain of apparently healthy mice. The intracellular formations seen in both rats and mice lead one to believe that at least in some cases their presence suggests latent virus.

PSEUDO SKIN REACTIONS AS A COMPLICATING FACTOR IN THE INTERPRETATION OF THE DICK REACTION BEFORE AND AFTER IMMUNIZATION AGAINST SCARLET FEVER. M. L. Menten and (by invitation) C. G. King and H. H. Finlay, Pittsburgh, Pa.

Abstract. Results of immunization against scarlet fever previously obtained by us in older individuals (doctors and nurses) indicated that in many of these despite 5 or even 6 immunizing doses of toxin many persistently remained Dick-positive, but were apparently not susceptible to scarlet fever. It appeared as if the skin of such individuals had acquired a sensitivity to certain proteins derived from the streptococcus.

In order to test such a hypothesis 112 individuals between the ages of 10 and 19 years, who gave positive skin reactions to Dick commercial toxin and specific toxin partially purified by us, were skin tested before immunization with the following 3 ancillary tests, *viz.*, toxin boiled $1\frac{1}{2}$ hours, dilute antitoxin, and toxin neutralized with antitoxin. They were then given at weekly intervals 5 immunizing doses. On retesting the following results were obtained: 55, or 49 per cent, were negative to Dick toxin and negative to our toxin; 34, or 30.4 per cent, were negative to Dick toxin and positive to our toxin; 23, or 20.5 per cent, were positive to Dick toxin and positive to our toxin. The 57 who showed positive reactions were then given the 3 ancillary tests. Fourteen of the group of 23, who after immunization were still positive to both tests, were originally negative to boiled toxin and through immunization had acquired a positive reaction to boiled toxin. Immunization procedures had apparently rendered the skin more sensitive to protein reaction. Further evidence of this phenomenon was also noted in the acquisition of sensitivity to antitoxin following immunization. Analysis of the other group of 34 gave similar data.

We believe the above may help to explain some of the discrepancies reported by investigators of immunization against scarlet fever.

HEREDITARY VARIATIONS IN THE BLOOD CYTOLOGY OF NORMAL RABBITS. Albert E. Casey, P. D. Rosahn, C. K. Hu (by invitation) and Louise Pearce, New York City.

Abstract. Studies in the laboratory of the Rockefeller Institute for Medical Research have demonstrated wide variations in the blood cell formulae of normal rabbits comparable in magnitude with variations in coat color, size, body and organ weights and other constitutional factors. Except for minor seasonal fluctuations these differing blood cell formulae were found to be fairly stable during conditions of health and vigor and to be closely related to the natural resistance of the rabbit host to several experimental and spontaneous diseases. Further studies on standard bred stock have revealed that the differences in the blood formulae among normal rabbits are largely inherited differences, and studies on the transmission of such characters are now being

made. The most striking evidence of inheritance was found in the values for the basophiles, lymphocytes, red blood cells, and the hemoglobin, factors which were found to be reliable indices of the natural resistance of rabbits to inoculation with a transmissible malignant tumor and with the spirochete of syphilis.

THE FATE OF INJECTED MARKED HOMOLOGOUS LEUKOCYTES IN THE GUINEA PIG. John Ungar and G. Randolph Wilson (by invitation), Pittsburgh, Pa.

Abstract. The purpose of the experiment was to observe the fate of marked homologous leukocytes injected into the circulation of the guinea pig. Leukocytes were obtained by the production of a chemical peritonitis with 5 per cent aleuronat. Carbon and a protein substance, carmine, were the suspensoid dyes injected for the purpose of marking the cells for later identification. Guinea pig serum injected together with the dye was shown to enhance phagocytosis both *in vivo* and *in vitro*. Several hours were allowed for phagocytosis. The abdomens were then opened and the cellular exudate removed and placed in a sterile flask which was kept in the dark at body temperature. It was then filtered. The filtrate was clear of free particulate matter and consisted of a pure suspension of leukocytes, approximately one-half of which contained phagocytosed dye particles. The cells were concentrated by slow centrifugalization and proved viable by their ability to phagocytose a contrasting dye *in vitro*. They were then injected intracardially in some animals and into the portal vein of others. Histological studies were made after various time intervals. The carbon-containing leukocytes were found to be concentrated in the lungs, free in the air spaces. Carmine-containing cells did not have a consistent distribution. Some appeared in the lungs to the exclusion of other organs, while in others destruction occurred chiefly in the liver.

Discussion

(Dr. N. C. Foot, New York City.) I do not think that any of us are so enthusiastic about the origin of the mononuclear cells in the intra-alveolar spaces from the blood as to maintain that all of them come from that tissue, but a piece of work like this is always very gratifying to those of us who have that belief. The mononuclears are so ubiquitous, they are such restless cells, and they move to and fro with such rapidity that it is very difficult to keep any track of them at all. This method of (shall we say bird-banding?) is a very satisfactory way of keeping track of them. There is one possibility, that these cells concentrate in the lung as they do on account of their large load of pigment. In other words, they act very much as pigment or particulate matter does, and I think it would be interesting to follow the experiment along and see if the cells remain viable in the lung for any length of time and whether or not they wander back from the alveolar cavity in the lymphatics.

THE CHEMOTROPIC ATTRACTION OF HUMAN LEUKOCYTES BY MICROÖRGANISMS AND VARIOUS SUBSTANCES. Morton McCutcheon, Harold M. Dixon (by invitation) and Edward B. Krumbhaar, Philadelphia, Pa.

Abstract. The chemotropic response of human polymorphonuclear leukocytes to different types of microörganisms was evaluated through experiments *in vitro*. Under the microscope the net distance was measured by which each cell approached a clump of bacteria, and this distance was divided by the total

length of the path of the cell. The resulting ratio is a measure of the chemotropic response, having as extreme values $+1.00$ if the cell moves directly toward the bacteria and -1.00 if the cell moves directly away from them. With staphylococci, streptococci, pneumococci, typhoid and tubercle bacilli, *Micrococcus tetragenus* and certain yeasts, mean ratios ranged only from $+0.73$ to $+0.86$, indicating approximately equal attraction under these conditions. With the yeast *Torula histolytica* the ratio was $+0.57$. Control leukocytes, wandering in fields free from known chemotropic influence, gave a value of $+0.07$. With substances other than bacteria a wide range of values was obtained: gelatin 0.00 ; dried blood $+0.18$; dried leukocytes $+0.25$; starch paste $+0.71$.

Discussion

(Dr. Louis Dienes, Boston.) Though it is well known, I should like to mention that the tubercle bacilli attract polymorphonuclear leukocytes also in the tissues, as in the diagram shown to us. The infiltration with mononuclear cells and the tubercle formation is a later reaction due, according to our observations, to the development of hypersensitiveness.

STUDY OF THE BONE MARROW IN APLASTIC ANEMIA. C. P. Rhoads and (by invitation) D. K. Miller, New York City.

Abstract. Twenty-two cases of so-called aplastic anemia have been studied both clinically and pathologically. These cases were divided into four subgroups according to the pathological changes observed in the bone marrow. Sternal marrow removed at biopsy was studied in all except 3 cases. In the first, or "classical aplastic" group, the marrows showed a striking reduction of total numbers of cells as well as an interference with maturation. In the second, or "immature" group, the marrows were cellular and hyperplastic and showed almost complete failure of maturation, the predominating cell being primitive in type. The third, or moderately mature group, showed cellular marrows in which the degree of failure of cell maturation was less than in the second group. The fourth group was one in which the marrow had been replaced by the tissue of Hodgkin's granuloma.

Supravital studies by the method of Sabin were made on all the biopsy material. These differential counts corresponded quite well with the appearance of the marrow as seen in sections. The similarity of the cell types present in the failure of maturation occurring in acute agranulocytosis to those occurring in the marrows of cases of aplastic anemia is a striking and important fact.

MYELOSARCOMATOSIS. Theodore R. Waugh, Montreal, Canada.

Abstract. The term myelosarcomatosis has been employed by various authors to designate atypical systemic myeloid processes. A review of these cases shows that they vary all the way from leukemic myeloses with tumor-like nodules to multiple myelomas with rather aggressive manner of growth. If any uniformity of nomenclature is to be preserved this term should be used to signify a process on the myeloid side, which is analogous to the relatively common lymphosarcomatosis on the lymphoid side. That such processes apparently occur is exemplified by the following case.

A male, aged 48, gave a clinical picture suggestive of Kahler's disease. There was marked Bence-Jones proteinuria and rarefaction of the bones. The blood

findings were essentially normal. Death resulted from pulmonary thrombosis. Autopsy revealed multiple nodules in the bone marrow with intervening fatty myeloid tissue. These nodules had thinned and expanded the cortex of the bones. There were no tumor masses in the internal organs but the spleen was moderately enlarged.

Histological examination of the nodules in the bone marrow showed areas of pleomorphic myeloid hyperplasia with comparatively few myeloblastic forms, and from these by gradual increase in the number of immature elements a transition into a typically neoplastic appearing type of proliferation. In some areas the dedifferentiation had reached such a degree that the cells presented the appearance of reticular elements. The splenic pulp was diffusely involved by similar immature cells and foci were present between the liver columns. The lymph glands apparently escaped.

It would appear, therefore, that there occurred primarily multiple focal areas of hyperplasia in the bone marrow with transformation to a neoplastic type of proliferation, and secondly, a systemic spread of this immature proliferative process to the spleen and liver. This type of reaction is distinctly analogous to that met with in many cases of lymphosarcomatosis, and hence would appear to justify the name myelosarcomatosis.

ANATOMICAL STUDIES ON PRIMARY AND POSTPRIMARY TUBERCULOSIS IN WHITE CHILDREN AND ADULTS. Kornel L. Terplan, Buffalo, N. Y.

Abstract. The anatomical incidence of tuberculosis among 312 white children between 1 month and 6 years of age was 4 per cent. Among 52 children and young adults ranging from 7 to 18 years of age it was about 20 per cent. In the first group 1 case showed one typical calcified Ghon focus in the lung parenchyma, first detected by postmortem radiographic examination, without complex formation. Similarly, one completely calcified small focal lesion was found in the 2nd case without changes in regional lymph nodes. (Histological serial sections were cut through all lymph nodes draining the sites of the calcified Ghon foci.)

Among 26 white adults, aged 20 to 40 years, 11 showed no signs of tuberculosis in lungs or intestines. (Postmortem roentgen films were taken and thorough macroscopic serial sections cut through both lungs and bronchomediastinal and mesenteric lymph nodes.)

Among 94 cases between 41 and 80 years of age only 4 were entirely free of tuberculous changes. In 7 cases (ages 38, 46, 55, 58, 58, 66 and 81 years) the anatomical findings pointed to a relatively late primary complex with still cheesy or cheesy fibrous changes. Although the primary foci were distinctly encapsulated calcification had not yet taken place. This feature was especially striking in a senile male of 82 years where the cheesy fibrous complex change was limited to two regional lymph nodes. With the exception of 1 case among these seven individuals, in which recent bronchogenic foci were present, spread from the primary focus or from the caseous lymph nodes to more centripetal lymph nodes or into the blood stream was not demonstrated. In 11 cases of the aged group under discussion distinct signs of an old primary focus or primary complex in a decidedly healed ossified or calcified ossified stage were observed, but in addition there were noted relatively recent cheesy, cheesy fibrous, or cheesy chalky fibrous, foci and complex changes in their regional lymph nodes which could not be demonstrably connected with the healed primary foci or

complexes. These last changes occurred in different areas of the lungs with separate lymphatic drainage; only in 1 case were they present in the same lobe as the healed lesion.

Conclusions: According to the postmortem figures at the Buffalo General and Children's Hospitals obtained from a systematic study over a period of 3 years primary tuberculous infection does not necessarily occur only in childhood. It is realized that the number of cases examined is too small to permit of definite statistical conclusion. Nevertheless, the anatomical results correspond approximately to the incidence of tuberculin-positive children and young adults between 10 and 18 years of age in the schools of Buffalo. The number of tuberculin-positive reactors lies between 20 and 25 per cent. From this study it seems likely that the primary focus or primary complex may be acquired very often in the years following puberty, *i.e.*, from the latter part of the second to the third and fourth decades. Evidence is at hand to warrant the belief that infections acquired after childhood may restrict themselves to focal and complex changes, as known in children, without producing active tuberculosis as a disease. In cases with anatomically healed Ghon foci or primary complexes, a second tuberculous infection may occur and produce again a so-called tuberculous complex with a focal lesion with involvement of regional lymph nodes. These second complexes point to true exogenous reinfection. Compared with the first healed complex they are by all available anatomical evidence relatively recent and decidedly younger.

Discussion

(Dr. Esmond R. Long, Philadelphia.) Dr. Terplan's results furnish interesting anatomical correlations with our epidemiological work. Twenty-five years ago in a city the size of Buffalo it would have been extraordinary to find an incidence of infection at the age of 40 as low as that given, with considerable increase later in life. He reported 60 per cent at that age, where formerly 95 per cent might have been expected. That probably represents the present situation throughout our own country. As a result of the antituberculosis campaign we are postponing the age of onset of primary tuberculosis. Our tuberculin tests at the present time show some such incidence as Dr. Terplan has indicated, but prior to this time we have not had good anatomical evidence that primary infections occur this late in life and pursue just the same course as they do when they occur in childhood. I think these results effectually dispose of that old belief that adults who escape primary lesions when they are children, such as people from the mountains and remote country districts, who come to the city late in life, are likely to break down with a massive primary tuberculosis. The actual fact is that if they develop primary tuberculosis it tends to pursue the same benign course as though they had developed it at the age of 5 or 6 years.

(Dr. Herbert S. Reichle, Cleveland.) The 8 cases of primary infection in the older age group are particularly interesting, but in addition to the entirely plausible view that these represent the first and only primary tuberculous infections in these individuals, there is just one other possibility which I think must be considered. I do not believe that the suggestion to be made is true in all cases; some, however, might be interpreted in this particular fashion. Huebschmann, who has been interested in tuberculosis for some years, pointed out that many primary infections have a central core of granulation tissue which

usually escapes our observation because we take but one section through the focus. Along the path of this granulation tissue true absorption may occur, not only of the calcium, but also of the bone, and thus a true primary focus may disappear entirely. I think he has proved his point in so far as it concerns the lung, but I do not know whether the same process is possible in the lymph nodes. It might be that in such cases the primary focus of childhood had already disappeared at a time that the second primary infection developed, which latter phenomenon has been discussed by Dr. Terplan.

(Dr. Terplan, closing.) I am, of course, familiar with Dr. Huebschmann's work on tuberculosis. However, I do not know whether he actually has proved the complete resorption of a primary Ghon focus in a child or young adult. Huebschmann believes that the first tuberculous lesion in the parenchyma of the lung is originally a pure exudative lesion of pneumonic nature, and he considers the possibility that even in this stage a resorption of the exudate may take place before the focus becomes caseous. In his opinion not even a small scar may remain. In my opinion this certainly would be, if at all possible, very exceptional from all that we know about primary tuberculous foci in man. I do not, however, question that small ossified lesions finally may be resorbed completely.

I saw in very rare cases and only in senile adults minute fragments of bone tissue in the lungs practically without any reaction in the nature of scar tissue surrounding them. One point I should have stressed a little more. In examining lungs from children and young adults for Ghon foci it was customary first to look for tuberculous changes in lymph nodes, and then to search for the focal lesions in those areas that were drained by the tuberculous lymph nodes. When the lymph nodes appeared normal it usually was taken for granted that there was no Ghon focus present, and these cases were considered negative. In 2 cases in my series, however, there were typical calcified Ghon foci, one of which was detected only by X-ray, and, still, complete serial sections through the lymph nodes regional to the Ghon foci did not reveal any tuberculosis.

It is therefore absolutely necessary to make routine roentgen photographs of lungs, especially in children and young adults, and, in addition, complete gross dissection of all lobes, before we feel sure that a case may be called negative.

THE HEALING OF TUBERCULOUS CAVITIES. A STUDY BY SERIAL SECTIONS.

Esmond R. Long and (by invitation) Gertrude Duetz, Philadelphia, Pa.

Abstract. In an autopsy on a patient dying from chronic ulcerative and fibroplastic pulmonary tuberculosis with extensive cavitation of the right lung a cavity was found in the left lower lobe which appeared to be healing. This cavity was 4 cm. in diameter and approximately spherical. Its outlet appeared to be extremely narrow and was pointed directly cranially. No fresh bronchogenic tuberculosis was found which could be related to dissemination of infectious material from this cavity, although the cavity was filled with a white semiliquid material containing numerous acid-fast bacilli. It appeared evident from the smallness of the outlet and the lack of surrounding infection that the cavity was not draining.

Serial sections were made of the neck of the cavity and the adjacent pulmonary tissue. In these sections several regions of healed, in some cases calcifying, bronchiogenic tuberculosis were found. One of these was connected with the cavity by a heavy band of scar tissue, in which traces of a lumen remained,

filled with degenerated cellular exudate. The calcifying mass at the distal end appeared to represent the inspissated contents of a cavity that had lost its outlet by scar tissue obliteration.

The outlet of the main cavity appeared to be undergoing a similar fate. It was lined by thick granulation tissue and filled with degenerating leukocytes. The diameter of the outlet at the cranial end was approximately 1 mm. The contents were so dense as to suggest that no movement leading to drainage could take place.

The entire system of healing tuberculous lesions appeared to have been due originally to bronchiogenic dissemination from the opposite lung. The caudocranial direction of the main and subsidiary outlets apparently slowed up drainage, after cavitation occurred, giving time for fibrosis of the outlets and further limitation of drainage. The study emphasizes impeding of drainage as the factor of chief importance in the healing of cavities. This factor is of clinical importance, not only in the common spontaneous healing of small cavities, but also in induced healing following artificial lung collapse. In the procedure the closure of soft-walled outlets of small cavities is probably of as much importance as pressure collapse of large cavities.

TUBERCULOSIS OF THE MAJOR BRONCHI. H. S. Reichle and (by invitation) T. T. Frost, Cleveland, Ohio.

Abstract. Tuberculosis of the major bronchi can be classified according to pathogenesis as (1) infection by implantation, (2) infection by contiguity, and (3) infection by continuity. The first is decidedly less common than the other two, a fact that may be ascribed to the protective influences of cilia, mucous and bronchial peristalsis. Infection by contiguity is prone to occur because of the proximity of the extracartilaginous mucous glands to diseased lymphatics and lymph nodes. Proliferation of the adventitia and atrophy of the mucous glands tend to close this avenue. Infection by continuity occurs secondarily to an implantation tuberculosis in the lower lobes and in the bronchi draining a tuberculous cavity. Major bronchi appear singularly resistant to tuberculosis; however, when fibrosis has transformed them into rigid tubes and mucosa and submucosa are destroyed and replaced by tuberculous granulation tissue, an open focus of disease is created which is rarely closed either by natural processes of healing or by pneumothorax or thoracoplasty.

Discussion

(Dr. Kornel Terplan, Buffalo.) We had occasion to see a case similar to Dr. Reichle's interesting observations in a completely healed stage. This case was that of a woman about 35 years of age with a known history of chronic tuberculosis many years previous to death. The clinical diagnosis was dextrocardia. She died suddenly from lobar pneumonia of the left lung. We found at post-mortem complete fibrous obliteration of the main bronchus affecting the entire lumen about 1 to 2 cm. distal to the carina. The surrounding tracheobronchial lymph nodes showed complete calcification. The obliteration of the main bronchus had, of course, produced complete collapse of the right lung with consequent shift of the mediastinum to the right and "dextrocardia."

POSTOPERATIVE LYCOPODIUM GRANULOMAS. I. H. Erb, Toronto, Canada.

Abstract. Six cases are reported in which there developed postoperatively a granulomatous lesion, the result of the introduction into the field of operation of numbers of lycopodium spores. For some years these spores have been used in many operating rooms as an ingredient of dusting powder for rubber gloves.

The lesion produced is of the nature of a foreign body granuloma and bears a striking resemblance to tuberculosis, with which it may be easily confused. As the spores are acid-fast, they present a striking picture in sections stained by the Ziehl-Neelsen method, which procedure is of value in differentiating the condition from tuberculosis.

The lesion is of importance, not only because it may result in intestinal obstruction through the formation of adhesions, but also because of the fact that, if unrecognized, it may lead to errors of diagnosis and thus to unnecessary surgical or other therapeutic measures.

It is the opinion of the author that the use of lycopodium spores as a dusting powder in operating rooms should be discontinued.

Discussion

(Dr. Ralph D. Lillie, Washington.) Shortly after the appearance of the article last fall we wanted some granulation tissue in large quantities so we thought we would try lycopodium in rats, but we made the mistake of sterilizing the lycopodium spores in a suspension of salt solution in the autoclave, and when we injected them into rats we did not get the tumors we had been led to expect. We made sections where they had been put in and could not find anything except a little yellow material. There was no particular granulation tissue reaction and the lycopodium spores which were there were no longer acid-fast. Just about that time I came down with influenza and went to bed, and when I got up I found too much encephalitis going on to follow it up. It seems to me, however, we must have hydrolyzed whatever it is that causes this foreign body reaction by autoclaving wet and under pressure.

(Dr. David A. Wood, San Francisco.) About 2 years ago I had the opportunity of seeing one of these lycopodium granulomas from a man 27 years of age who had had a laparotomy 3 months before. In routine stains the lycopodium spores were refractile. The people in the West are looking for various mycotic infections, and it is a good point to keep in mind that one should look at lycopodium granulomas carefully, because occasionally some spores are oval and, in the routine stains, one might easily mistake them for the empty spores of *Coccidiodes immitis*. In my particular case the giant cells were very beautiful with many of the lycopodium spores inside. So the matter of mycotic infections is just another point to be kept in mind in the differential diagnosis.

(Dr. Erb.) In reply to what Dr. Lillie said, we did not try sterilization of any of these spores in saline or other fluid. Of course in the operating room they are always sterilized dry, but under pressure. We did, however, inoculate some guinea pigs, and we thought we were going to get beautiful lesions but we were disappointed. I thought we would get little nodules all over the peritoneum, but the spores were all picked up in the lymph nodes where they were surrounded by foreign body giant cells. We did not find the typical concentric arrangement.

(Dr. Howard T. Karsner, Cleveland.) In an attempt to produce pulmonary hypertension we have injected lycopodium spores intravenously into dogs. The

animals have survived for more than a year. The presence of the spores in the arterioles and capillaries is accompanied by the development of small granulomatous masses but without multinucleated giant cells, perhaps because the spores are within the vessels.

GRANULOMATOUS MYOCARDITIS. A CASE FOR DIAGNOSIS. James Miller, Kingston, Ontario.

Abstract. A series of cases of myocarditis of a subacute type and of doubtful origin has been reported in the literature from time to time. It is uncertain if these can be regarded as being due to one and the same causal germ. Most have been thought to be tuberculous, but complete proof has usually been wanting. The following case seems to fall into the category of subacute myocarditis of bacterial origin due to some unknown virus.

A Chinese, after showing a series of metastatic inflammatory foci in different parts of the body during a period of some months, died of acute peritonitis, pericarditis and myocarditis. The heart showed a diffuse myocarditis with extensive areas of necrosis and hemorrhage in the wall of the left ventricle. Microscopically the condition was definitely inflammatory and the cells were predominantly mononuclear. No organisms could be found either microscopically or on culture, and there were no spirochetes or tubercle bacilli. There was a single ulcer in the small bowel, transverse in direction, with raised margins. It seems probable that this may have been the origin of the peritonitis. The pericarditis was accounted for by the underlying inflammation of the heart muscle. There was no lesion of the coronary artery.

This case falls into the category of subacute myocarditis on account of the predominance of mononuclear cells in the areas of infiltration. There was no histological support for the view that it was tuberculous, apart from the extensive necrosis. There was scarring at the apex of the left lung and there was one ulcer of the small bowel, grossly suggestive of a tuberculous lesion, but the microscope revealed the same type of cellular change as in the heart muscle. One metastatic focus of infection was examined during the life of the patient but it also showed changes of a non-specific type.

In view of the absence of proof of the nature of the causal germ the term "granulomatous myocarditis" is suggested as covering best the histological and gross characteristics of the case.

Discussion

(Dr. Howard T. Karsner, Cleveland.) Problems such as presented by this case are of profound interest. Having seen the specimen and heard the presentation several questions arise. It is not necessary to exclude syphilis as a cause by virtue of the negative Wassermann test and the inability to demonstrate spirochetes. There are, however, no clearly positive stigmata of syphilis. The number of instances of isolated acute myocarditis of Fiedler is small and nothing is known of those cases which may perhaps progress to healing. The extensive necrosis in Dr. Miller's case is not duplicated in isolated acute myocarditis and in this latter condition hypertrophy appears to be constant. The condition in this heart might possibly be attributed to coronary disease, for although the larger coronaries were normal, it is still possible that examination of the minute twigs of the coronaries might show significant disease in that situation. I have

observed multiple small areas of fibrosis in the myocardium, by no means identical with the granulomatous lesion of this case, in hearts without gross coronary disease but with extensive sclerosis of small twigs.

(Dr. Ralph D. Lillie, Washington.) Something about this case recalled to my mind the lesions we have been studying recently in experimental subacute tularemia in animals. There is in that disease, not infrequently, a granulomatous myocarditis with peritoneal involvement which may be granulomatous. I offer this merely as a possibility. We do not know all about human tularemia yet.

(Dr. Miller.) I agree that a negative Wassermann does not exclude syphilis. I am not familiar with the work of Fiedler and I hope Dr. Karsner will give me the reference.

As to what Dr. Lillie said about tularemia, that did enter my head as a possibility, but with a history such as this, lasting 8 months, it seemed highly unlikely.

(Dr. Lillie.) It is not usual, but there have been a few cases which have gone on for 1 or 2, or even more, years.

(Dr. Miller, closing.) That is a matter which one might take further into consideration.

RADIAL INCLUSIONS OF GIANT CELLS. Edwin F. Hirsch, Chicago, Ill.

Abstract. Giant cells with rosette inclusions have been observed in lesions simulating tuberculous or foreign body granulation tissues. The nature of these inclusions has been an enigma and many divergent opinions have been recorded regarding their composition and the significance of the associated lesions. My observance of these granulation tissues has been in the lungs, spleen or lymph nodes of 8 bodies postmortem, and in 26 tissues removed surgically. The most conspicuous lesions observed in the postmortem material were in the spleen, lungs and parabronchial lymph nodes of a man, aged 72 years, who for many years had had urinary concretions, pyelitis and cystitis. He had taken one ounce doses of olive oil, presumably for some time. His spleen weighed 290 gm. and had many discrete fibrous nodules several mm. to 2 cm. in diameter. By chemical extraction the spleen contained quantities of fat melting at 65° C to 75° C and, when cool, containing rosettes like the inclusions and the crystals of palmitin or stearin.

All authors state that the radial inclusions in the giant cells are colored by nuclear and elastin stains, especially by the purple of Mallory's phosphotungstic acid hematoxylin stain. Wolbach and others have described tinctorial differences indicating that the inclusion material may be a mixture. The inclusions do not react with fat stains. Their configuration is crystalline and therefore they form in tissues according to the usual laws governing crystallization. A crystalloid in an aqueous system, according to Schade, separates in concentrically laminated spheres in the presence of small amounts of colloiddally dispersed protein. Theoretically, at least, the deduction follows that the radial inclusions of the giant cells separated from a solvent not aqueous.

Lipins stimulate foreign body tissue reactions, notably cholesterol. Intravenous injections of palmitin and stearin in olein and of soaps of calcium or magnesium in olein failed to produce the characteristic lesions with giant cells. Mixtures of stearin and palmitin and cholesterol in olein injected intravenously into rabbits produced lesions in the lungs comparable to those observed

in human tissues, except that the crystalline fat inclusions were not completely reproduced. That is, only the peripheral portions, the spines, of the crystalline fat had become insoluble. Such portions stained according to the descriptions given for the radial inclusion.

These experiments, of course, indicate, simply, that a lipin mixture containing cholesterol may produce tubercle-like lesions containing giant cells and that crystals of palmitin and stearin or their derivatives may become insoluble rosette inclusions of the giant cells.

ETIOLOGY OF CONGENITAL BILATERAL POLYCYSTIC KIDNEYS. James E. Davis, Detroit, Mich.

Abstract. Illustrations of the evidences of tissue deficiencies are apparent in the quantity and quality of the structures of the congenital bilateral polycystic kidneys at all ages. The stroma in these kidneys, particularly that in proximity with the immature epithelial parts, is of mesothelial character. This observation can be confirmed in kidneys from patients over 50 years of age. The quantity of epithelial tissue present in both kidneys is markedly deficient. Part of this deficiency may be inherited and part acquired through the influence of cystic degenerative changes. The inherent growth impulse of the epithelial tissue is inadequate and unbalanced. There are numerous contrasts of unassembled, partly assembled and completely assembled epithelial cells. The assembled cells exhibit the characteristic diffusely appearing cystic degenerative change. The arrangement of the unit parts of this type of kidney does not enable a conclusive opinion whether there is here strictly a metanephric condition of defectiveness or not.

Discussion

(Dr. Kornel Terplan, Buffalo.) I should like to ask Dr. Davis whether he has observed any hyperplastic changes in small arteries and arterioles in those polycystic kidneys in stillborn and very young infants in which practically no normal parenchyma had remained. I saw such changes in markedly hypoplastic kidneys in young infants.

(Dr. Davis, closing.) I have not observed any hyperplastic changes in the arteries.

DISAPPEARANCE OF GLOMERULI IN CHRONIC KIDNEY DISEASE. Alan R. Moritz and (by invitation) J. M. Hayman, Jr., Cleveland.

Abstract. The total number of patent glomeruli in a series of normal and abnormal kidneys was determined by Kunkel's modification of Vimtrup's method. Before the kidneys were macerated for counting the number of injected glomeruli blocks were taken for histological examination and the ratio of injected, uninjected and obliterated glomeruli was determined. From this ratio and the total number of injected glomeruli the total number of recognizable glomerular structures was calculated. This total included all scars that might possibly represent glomeruli. It was found that in arteriolar nephrosclerosis, chronic diffuse glomerulonephritis and chronic pyelonephritis a large proportion of the glomeruli may disappear completely.

The mode of absorption of the glomerular scars was studied in uninterrupted series of sections. It was found that the hyalinized spherical scar of the obliter-

ated glomerulus is penetrated by argentophilic fibrils which eventually become continuous with and conform to the pattern of the interstitial reticulum of the kidney. The hyalin undergoes peripheral and central rarefaction and disappears.

Experimental glomerular damage was produced by unilateral X-ray exposure of temporarily exteriorized kidneys of rabbits. Complete disappearance of a large proportion of glomeruli was observed following such treatment.

It was concluded that since glomerular scars disappear completely less information as to the severity and type of renal injury may be obtained from the microscopic examination of kidneys from cases of chronic Bright's disease than is commonly supposed.

THE MORPHOLOGY OF THE SENILE PROSTATE. Robert A. Moore, New York City.

Abstract. The morphology of the normal prostate varies with the age of the individual. In senility, as contrasted with young adulthood, there is a decrease in the height of the epithelium, a decrease in the size of the glands and a loss of the papillary infoldings. In association with these epithelial changes there is a fibrosis of the stroma, both intra- and interlobular, and a corresponding decrease in smooth muscle fibers. By comparison with the experimental effects of gonadectomy it would seem probable that these alterations are related to a decrease in the male sex hormone output of the testes. If there be an associated arteriolar sclerosis the acini undergo a peculiar type of complete atrophy.

Discussion

(Dr. Alan R. Moritz, Cleveland.) I should like to know if the arteriolar change is part of a systemic arteriolar sclerosis or if it is an isolated lesion, as seen in the senile uterus and ovary.

(Dr. Moore.) This series of prostates was taken from Dr. Erdheim's service, where there were no microscopic examinations, but, in the few cases in which there was a record in the autopsy protocol of nephrosclerosis, this type of process occurred in the prostate. It is my own belief from the few cases where I have complete material at Western Reserve and Cornell that this change is a part of a generalized arteriolar disease and is not a functional process, such as occurs in the uterus and ovary.

THE ISSUES AT STAKE IN THE GRADING OF TUMORS. Stanley P. Reimann and (by invitation) Clark E. Brown, Philadelphia, Pa.

Abstract. Two hundred cases of carcinoma of the breast have been graded histologically and their subsequent histories determined. The biological principles which are either consciously or unconsciously evoked when attempts at tumor grading are made are discussed, and the conclusion is drawn that cellular growth is too dynamic a process and the variables are too numerous for a static bit of evidence, like histological slides, to be of use in the prognosis of any individual case.

Discussion

(Dr. William Carpenter MacCarty, Rochester, Minn.) I should like to make three points in connection with this subject. First, I want to commend the speaker on his conservatism, with which I thoroughly agree.

In the second place, I think probably I am one of the most active individuals who have studied this problem. I am one of the few individuals who have written much about it. In my opinion grading has only biological significance. It is of interest to those of us who are interested in the behavior of cancer, and I may say that is my particular interest in the subject.

The third point is the lack of specificity in the percentages. Suppose I said to our President here: "You have a Grade 4 cancer," which I hope he has not. I am the pathologist talking to the surgeon. He will say to me: "Dr. MacCarty, what is the prognosis?" and I will say, "I will give you the percentages." Suppose I tell him that in Grade 4 cancer in 80 per cent of the cases death takes place in say 2 years, and that in 20 per cent they live longer than that, 5 years, let us say. My friend will then say: "You tell me I have a Grade 4 cancer, and you also say that 80 per cent live 2 years, and 20 per cent live 5 years, or longer, but I am particularly interested in myself — how long am I going to live? That is what I want to know." Then I will have to fold up my wings and drop my tail and say, "I am awfully sorry; I am a scientist but I cannot tell you that." If I were a surgeon, and I presume I have seen more surgery than any surgeon, there would be one main criterion by which I would decide to remove a tumor, benign or malignant. I would first decide whether the patient was in good condition — heart, kidneys, tissue fluids, age and so forth. I would then decide whether there was any glandular involvement which I could not reach, and after I had decided I could remove the tumor and glands I would do so, and I would not care what grade it was. I have seen patients with Grade 4 tumors, the worst grade, live many years, even in spite of the fact that they have had glandular involvement, simply because the growth was easily accessible and the surgeon did a radical job. I want to emphasize that I think this grading has biological interest more than it has practical importance, so far as the individual is concerned.

(Dr. Shields Warren, Boston.) I have been interested in grading, not so long a time as Dr. MacCarty has, but I have profited very largely by his example and advice, and have also profited extensively by Dr. Reimann's work in the field of the breast.

I wish to emphasize one point that was made by Dr. Reimann and Dr. Brown — that the environment is a very important factor. In the study of the material that I presented yesterday it was noticeable in relation to age, known to be an important factor in determining clinical malignancy (that is, young people in general doing more poorly with a given tumor of a given extent than older people), that there was no difference in the grade of the tumors in a young person as against those in an older one. The percentage ran about the same. The other point which I think all pathologists who have to deal with surgeons have run up against is the temptation in the lower grades for the surgeon to attempt a less radical operative procedure. I feel that, if we do grade tumors, we must also constantly remind those who depend on our diagnosis that cancer is cancer, whether it is Grade 1 or Grade 4.

(Dr. Brown, closing.) I should like to thank Dr. MacCarty and Dr. Warren for this constructive criticism, and I am sure we are in hearty accord with everything they have said. I might also bring to mind in closing, drawing from the rich elocutionary achievements of last night's dinner performance, that contention as well as hope springs eternal in the human breast. Hope certainly is needed in dealing with the biological fundamentals of cancer.

MELANOBLASTS OF THE ANAL CANAL. THEIR RELATION TO PRIMARY MELANOMA OF THE RECTUM. George F. Laidlaw and (by invitation) Charles L. Janssen and A. Purdy Stout, New York City.

Abstract. The anal canal is lined with stratified epithelium of ectodermal origin. This epithelium contains abundant melanin and melanoblasts up to the mucocutaneous line. Above this line there are no melanoblasts and no melanin. Here the mucosa consists of tubular glands of endodermal origin. It is probable that primary melanoma of the rectum arises from the melanoblasts of the anal canal.

Discussion

(Dr. Shields Warren, Boston.) I should like to ask Dr. Laidlaw what he regards to be the mode of production of the submucous pigmentation in the so-called melanosis coli.

(Dr. B. Earl Clarke, Providence.) A year ago I reported before this Association a tumor which, as nearly as we could determine, was primary in the upper jejunum. At that time I found in the literature 1 other case of primary melanoma of the jejunum reported by Dr. Lund of Boston. Has Dr. Laidlaw made any study of melanin-producing cells in the small intestine, and what is his opinion as to the mode of origin of these tumors?

(Dr. Ernest M. Hall, Los Angeles.) Two cases of supposed primary melanoma of the small intestine were discussed at a recent meeting of the Los Angeles Pathological Society. One of these tumors, reported from the Los Angeles General Hospital, was the size of a walnut; the other, reported by Dr. Foord from the Pasadena Hospital, was the size of a man's fist. There were two tumors present, in the latter case, of almost equal size and no melanomas could be found elsewhere.

(Dr. Victor C. Jacobsen, Albany.) I have been studying melanin for many years and trying to see if we can explain melanin, wherever it is found in the human, as originating chiefly in the neuro-ectodermal cells. Almost all of it can be so explained. There are a few exceptions and Dr. Laidlaw has mentioned them. One condition that has given difficulty is melanosis coli. The colon in extreme cases of constipation or of chronic obstruction low down may become intensely pigmented with true melanin. The melanin is present in cells in the mucosa beneath an intact epithelial lining. These cells are "dopa"-negative. Hence they do not make melanin; they simply phagocyte it. The question is, where does it come from? In studying the excretion of melanin in the body the kidney has been proved to be an important source of excretion, and of course the skin by desquamation. A certain amount must escape into the intestine along with foodstuffs of animal and vegetable nature which contain melanin. It is quite apparent that in the intestine are all the precursor substances necessary to form that molecule of unknown composition which is melanin. It is very probable that there is an actual synthesis of melanin in the intestine, particularly in constipated people, and that its presence in melanosis coli merely means that it is being phagocyted and not actually made by the cells which contain it.

(Dr. Ralph D. Lillie, Washington.) In regard to melanosis of the colon in minor degrees, it is quite frequent, according to Henschen and Bergstrand. We did some work with it in the appendix in Washington. It appears to me that melanin is finally formed in the large round phagocytes immediately beneath the epithelium, because in quite a number of cases we have seen granular material

not staining like true melanin and occurring first as fine unpigmented granules, then as finely granular brown pigment in the large round cells just under the epithelium.

(Dr. Laidlaw.) Beginning with the last question, Dr. Lillie says "staining like true melanin." What is the criterion for staining like true melanin?

(Dr. Lillie.) The melanin which is stained in melanomas takes an olive green color with toluidin blue, and we find all gradings from a very faint blue color into a dirty olive green in the cells immediately under the epithelium in melanosis of the appendix.

(Dr. Laidlaw.) In forming conclusions from staining reactions we should bear in mind that the thing we call melanin does not always stain uniformly. Dr. Lillie's observation of the behavior of melanin in the appendix is paralleled in silver staining of pigmented skin. If a section of such skin be dipped in silver the melanin in the epidermis will turn black in 5 seconds, but the melanin in the derma will show all grades of staining from black to no staining at all. After several hours or possibly several days in silver all of the melanin granules in the derma will be black. Nevertheless, we call all of these granules melanin, whether they stain slowly or rapidly. It is our experience that intestinal melanin is particularly difficult to stain, sometimes requiring hot silver and many hours to secure uniform staining. It seems to me that Dr. Lillie's brown granules in the appendiceal mucosa are ordinary intestinal melanin giving the customary phenomenon of different degrees of color reaction.

(Dr. Lillie.) We relied on the insolubility in mineral acids, the tinging with basic anilin dyes and the negative iron reaction, making three criteria, and used silver in relatively few cases.

(Dr. Laidlaw.) In regard to melanosis coli, the first question is this — Was the melanin manufactured on the spot by the cell which contains it, or was it merely phagocyted? The best answer to this question is a positive or negative "dopa" reaction. An important precaution should be noted here. A "dopa" reaction must be carried out on fresh tissue. Autopsy material secured many hours after death is unsuitable and uniformly gives a negative reaction. We have been fortunate in securing fresh specimens of melanosis recti and one of melanosis coli from surgical operations. There was abundant melanin in the mucosa but the "dopa" reactions were uniformly negative. We conclude, therefore, that the melanin was not made by the cell which contains it but that it was phagocyted. If phagocyted, where did the melanin come from? The most likely source is the contents of the intestinal canal. Melanin occurs in so many foodstuffs, both animal and vegetable, that it may easily be present in the food refuse of the intestinal canal. The association of melanosis coli with chronic constipation and obstruction would seem to be explained by prolonged contact permitting better absorption of the melanin. I admit freely that these simple mechanical explanations leave something to be desired. The question of melanosis coli, like that of most melanoses in the human body, has not received its final explanation.

Returning to the questions of Dr. Clarke and Dr. Hall, the supposed occurrence of primary melanoma in the upper intestine where normally no melanoblasts have been found presents a problem for which I do not yet know the answer. Possibly the answer is that of Oberndorfer that such tumors are really secondary to a primary tumor which has been overlooked. As a working hypothesis, in our laboratory we believe that in all probability primary melanoma appears only where melanoblasts already exist.

A MALIGNANT HEMANGIOMA WITH METASTASES. E. M. Hall, Los Angeles, Cal.

Abstract. The metastasizing hemangiomas are not only rare tumors but are also interesting because of their relation to the general malignancy problem. All autopsied cases should, therefore, be recorded in the literature.

In the case to be reported the patient was a well developed, white female, married, and 40 years of age. She had felt weak and exhausted for a period of 3 months. She became markedly anemic (hemoglobin 30 per cent) and dyspneic, with rapid pulse and low blood pressure. The presence of a cervical polyp suggested uterine bleeding as the cause of her anemia. She developed a massive right-sided hemothorax and died.

The autopsy, performed 3½ hours after death, revealed a well developed white female with marked pallor of the skin and mucous membranes. There were no angiomatous or pigmented moles anywhere in the skin.

The right pleural cavity contained about 3000 cc. of bloody fluid, while the left pleura contained nearly a liter of blood-stained fluid. The pericardium and heart were normal except for an abnormality of the right coronary artery which showed a small dimple in the aorta at the usual site of origin, opposite the right cusp, but no opening into the vessel. The coronary artery was present, but only about one-half normal size.

The right lung was almost completely collapsed and about the size of a man's fist. A number of small, dark, blood-filled tumors were present in the pleura, in the lower portion of the upper lobe and throughout the lower lobes. Along the lower border of the lower lobe there was a firm reddish mass 5 by 2 by 1.5 cm., with a roughened surface. This mass was composed of angiomatous tumor surrounded by hemorrhage, and was likewise the source of the bloody pleural effusion. The left lung was only partially collapsed. There were about twenty similar angiomas in the pleural surfaces, with two larger nodules present, the largest measuring 2 by 1.5 by 1.5 cm. A few angiomatous areas were found deep in the lung parenchyma, but most of them were subpleural.

The spleen, adrenals and kidneys were normal. The liver contained a number of mottled, reddish gray tumor nodules 1 to 1.5 cm. in diameter. These had the appearance of vascular carcinomatous metastases. The largest of these was on the under surface of the right lobe, measured 2.5 cm. in diameter, and was somewhat raised above the surface of the liver.

A number of moderately swollen hemorrhagic lymph nodes bordered the pancreas and a firm mass the size of a man's fist was found immediately below the pancreas in the midline. On cutting through this mass it was found to consist of fibrofatty tissue surrounding a group of 13 to 15 enlarged hemorrhagic lymph nodes. Similar nodes were found along the aorta as far as the bifurcation.

The aorta appeared somewhat smaller than normal. The leptomeninges and the brain substance were very pale. The vessels at the base of the brain were considerably smaller than normal. The bone marrow of the sternum was pale red.

Histological examination of one of the tumor nodules (lymph node) shows one part consisting of large, thin-walled spaces filled with red blood cells and lined by flattened endothelium. The other portion of the gland is cellular, consisting of small, irregular or collapsed spaces lined by large atypical cells varying from fusiform to large polygonal. In the latter the nuclei are oval or spherical, many are hyperchromatic and occasional mitoses are seen. The cellular portion has a distinctly malignant appearance.

The sections of the lung show quite a similar picture, except that the cavernous spaces are partly confluent and in several places thrombosed, with more or less hemorrhage into the surrounding tissue. One of the sections from the liver shows a fairly large vein, the wall of which is invaded by the tumor with irregular masses of tumor cells within the lumen.

The primary tumor was probably in the right lung, although one cannot say with any great degree of certainty. The tumors of the liver were decidedly like metastases in appearance. It is evident that the tumor was histologically malignant, as well as producing widespread metastases. Evidence of congenital vascular deficiency is shown in the presence of an imperforate right coronary and in hypoplasia of this vessel, together with the cerebral vessels and the aorta.

Discussion

(Dr. Kornel E. Terplan, Buffalo.) I should like to ask Dr. Hall whether the lymph nodes draining the lungs were involved. If the bronchomediastinal chain was not involved it may be well to consider whether the primary site of the tumor was not in the retroperitoneal lymph nodes. I happened to see in Prague 2 cases of malignant hemangioblastoma which apparently originated in the retroperitoneal lymph nodes, producing extensive metastases in the liver. One of these cases had also metastasized to the lungs.

(Dr. Hall, closing.) I am very much interested to hear what Dr. Terplan has to say. The question in my mind was this — whether the tumor was primary in the retroperitoneal lymph nodes or in the right lung. The lymph nodes in the mediastinal chain were not involved in this case. Also, I might add that the pain in the right lumbar region and in the right hip could be accounted for by the tumor mass below the pancreas.

SUPERIOR PULMONARY SULCUS TUMOR (PANCOAST). B. Earl Clarke, Providence, R. I.

Abstract. Pancoast in 1932 reported 7 cases which he believed to represent a new disease entity and for which he proposed the name of "superior pulmonary sulcus tumor." He predicates such a diagnosis upon the following findings: (1) pain around the shoulder and down the arm; (2) loss of power and wasting of the muscles of the hand; (3) the presence of a Horner's syndrome; and (4) a characteristic roentgenographic picture of "a comparatively small and circumscribed shadow in the apex, due to lung displacement, together with destruction of the posterior portions of one or more ribs and the adjacent articular and transverse processes and possibly involvement of the bodies of one or more vertebrae."

None of Pancoast's 7 cases came to autopsy. In 2 there was surgical intervention and tissue was obtained for histological examination. The 1st of these, operated upon in 1921, was originally said to be an endothelioma. However, a slide from this case was seen by Dr. Joseph McFarland, and in his opinion the histology is that of a "carcinoma spinocellulare." In a 2nd case, operated upon in 1922, the histological diagnosis was carcinoma. Apparently no slide was available for study. So far as I can learn the postmortem findings in such a case have never been recorded in the literature.

Our patient, a white male, 53 years of age, entered the Rhode Island Hospital Sept. 19, 1933. He presented clinical and X-ray findings in every way typical of those described by Pancoast (lantern slides of roentgenographs shown).

At postmortem examination the apex of the left lung was found adherent to the underlying tumor mass. When pulled away it left a flattened area on the posterior aspect of the apex. Tumor tissue was found to extend into the lung substance only 0.5 cm. beneath this flattened surface.

The tumor extended around the posterior aspect of the upper thoracic cavity destroying the posterior 3 inches of the left third rib and about 1 inch of the left second rib. It extended across the midline destroying the entire body of the second dorsal vertebra and parts of the bodies of the first and third vertebrae. The tumor pressed upon the spinal cord but the dura was not invaded. A small portion of the right second rib was destroyed. The apex of the right lung was not adherent. The nerve roots and the posterior root ganglia were lost in the tumor.

The histology of the tumor is typically that of an epidermoid carcinoma. In the more differentiated portions the cells occur in rather large sheets and have the characteristics and arrangement of prickle cells. In a few places actual protoplasmic bridges are demonstrable. There is no keratinization. Other parts are poorly differentiated, but the cells are thought always to be epithelial in type (photomicrographs shown).

The origin of this tumor is not entirely clear. Three possible sources are to be considered. We include the pleura because the original histological diagnosis of Pancoast's first case was "endothelioma." Pancoast asks why such a growth in this one location should always invade bone and never elsewhere? McFarland rejects the histological diagnosis of endothelioma. Surely my case cannot be of pleural origin. I think we can rule out the pleura.

The possibility of primary carcinoma of the lung cannot be so lightly passed over. We know that tumors of similar histology do originate in the lung. McFarland (quoted by Pancoast) points out that tumors of this cell type are not so apt to be found in an apical lung lesion so far away from the larger bronchi. Again we might ask why, in this one location, should a primary lung tumor always invade bone and never elsewhere? It seems to me highly improbable that a tumor of the lung with such meager lung involvement could be primary to such extensive extrapulmonary extension. In spite of all this I am loath to reject this possibility.

A third possibility suggested by Pancoast is an origin from an embryonic rest, similar to that of branchiogenic carcinoma. He postulates such an origin from the fifth pharyngeal pouch (ultimobranchial body). While such a possibility cannot be denied I see no way of establishing its correctness.

I must confess, then, that I am unable to arrive at a definite decision as to the origin of this peculiar tumor. It is quite possible that no one of the above suggestions is correct. It may even be that this is not a pathological entity. Tumors of different histological types in this particular location might produce the same clinical and roentgenological findings.

Discussion

(Dr. Howard T. Karsner, Cleveland.) At this time I wish to place on record another case in which the X-ray findings were those of superior pulmonary sulcus tumor. The patient died after having symptoms and signs for 4 months. At the autopsy a tumor was found in the right superior pulmonary sulcus, involving the lower part of the neck and the upper part of the right lung. This eroded bones locally and was accompanied by metastases to the right adrenal,

right kidney and intestinal tract. The tumor has not been completely studied microscopically, but at the present time a tentative diagnosis of sympathicoblastoma is presented. The tumor was not in a situation to have arisen from the carotid body and presumably is of sympathetic system origin.

(Dr. Paul G. Weston, Jamestown, N. Y.) Some months ago I found a tumor at autopsy in exactly the same site as that described by Dr. Clarke, but it extended back to the foramen into the spinal canal and had its origin from the spinal dura. Grossly it was a fairly well circumscribed tumor, about the size of one's fist, had displaced the esophagus, as Dr. Karsner's case had, but did not show any metastases anywhere. The histology was that of a typical endothelioma starting in the dura of the cord. I was unable to obtain the clinical history because it was a coroner's case.

(Dr. Clark E. Brown, Philadelphia.) Recently we have had a postmortem on a case very similar to the one described by Dr. Karsner in which there was what we thought to be the original tumor in the apex of the right lung, or in the subclavicular fossa on the right side; it was impossible to say which. It was very destructive in nature and consisted of a soupy, grayish material. What we took to be metastatic involvement of both adrenals was found, and also involvement of the mesenteric nodes and the submucous tissue of the stomach. Histologically the predominant cells were multinucleated, pear-shaped and uninnuclear spindle-shaped cells, which we took to be those of spongioblastoma multiforme. We had some discussion with Dr. McFarland about this tumor and he was inclined to agree with that diagnosis at first, but on further thought he showed us some sections of epidermoid carcinoma which looked very much like the cells of this tumor.

(Dr. William Carpenter MacCarty, Rochester, Minn.) Was the posterior nares examined in this case? I have seen several intrathoracic tumors where the primary tumor was in the posterior nasopharynx.

(Dr. Victor C. Jacobsen, Albany.) I did not realize that this tumor was so rare. One case, which I have included in an increasingly large series of primary carcinomas of the lung, is similar to the case reported and very much like Dr. Karsner's. X-ray showed invasion of the ribs and vertebrae by the tumor. I made the first diagnosis of epidermoid carcinoma from biopsy material removed from just alongside the vertebra. At autopsy the tumor was present in the right upper lobe, toward the midline. I took the lung out carefully and injected it with Kaiserling solution. Later, when it was well fixed, I dissected out the bronchi leading to the region of the tumor and found the right eparterial bronchus disappearing in the tumor. Sections at that point showed a transition from bronchial epithelium to the squamous epithelium of this type of cancer. I have included it as a case of epidermoid carcinoma of the bronchus, in which category about 32 per cent of all lung carcinomas belong.

(Dr. Kornel L. Terplan, Buffalo.) With regard to Dr. Karsner's discussion, I should like to mention that malignant neuroblastic tumors of the peripheric nerve, especially of the sympathetic nerve, are very unusual in adults. A possible neurogenic origin, however, may be suggested by rosette-like arrangements of the immature blastoma cells, if we fail to demonstrate by all specific methods any more mature nervous element. Was there such an arrangement in your case, Dr. Karsner? Otherwise, I believe that Dr. Clarke's tumor may have originated from the pleura or from the lung itself. Sometimes carcinomas of the lung may grow into the pleural cavity, similar to a mushroom, and spread along the pleural surface. On the other hand, primary tumors of the pleura may differentiate into

epithelial tumors, as well as more immature mesenchymal or mesothelial tumors.

(Dr. Paul R. Cannon, Chicago.) I wish to mention the occurrence of one of these types of tumors in an autopsy performed by Dr. Steiner of the University of Chicago. The patient had a tumor in the left pulmonary sulcus with a Horner's syndrome and roentgenological findings consistent with a diagnosis of pulmonary sulcus tumor. Postmortem examination, however, revealed a typical adenocarcinoma, rich in mucin, located in the left pulmonary apex and extending into the pleura. Metastases were also present in the pelvic bones. Dissection of the primary growth suggested that the tumor had originated in an area of fibroplastic tuberculosis in the apex and we concluded that the tumor was a bronchogenic carcinoma.

(Dr. Clarke, closing.) This discussion seems to support my closing suggestion that the lesion is not a pathological entity. So far as the histological diagnosis in my case is concerned, sections were studied by Dr. Ewing, Dr. Mallory and Dr. Wolbach. They all arrived at the same diagnosis, epidermoid carcinoma, although they did not agree as to where it might originate. On my way here I stopped at Montreal and the sections were seen by Dr. Vernon Cone, who was of the opinion it did not originate in any nervous structure.

In answer to Dr. MacCarty's question, the nasopharynx was examined.

I was unable in this case to trace any connection with the bronchus. The lung involvement was so superficial that only a very small bronchiole could possibly have been involved.

PINEAL TERATOMA WITH UNUSUAL ADAMANTINOUS FEATURES. Samuel Judd Bochner and John E. Scarff (by invitation), New York City.

Abstract. Pineal teratomas are rare, only 14 completed cases having been reported, of which only 2 are in the American literature. Another case is here added, presenting unique dentigenous features hitherto not reported in the literature.

J. S., a boy of 9 years, was admitted to the Pediatric Service of the New York Post-Graduate Hospital with the history that for the past 2 years he had suffered occasional attacks of sharply localized frontal headaches, associated with extreme drowsiness and followed by apparent complete recovery. On the morning prior to admission a more severe attack occurred, accompanied by convulsive tremors and unconsciousness. On the ward he suffered another such seizure, associated with irregularity of the pulse, Cheyne-Stokes' respiration and a semi-comatose state terminating in death.

The unusual X-rays first attracted attention to this case. These seemed to show well advanced tooth structures in the region of the third ventricle.

Autopsy revealed a semicystic tumor filling and distending the third ventricle and markedly compressing the adjoining structures. It measured 5 by 5 by 3.5 cm. Section revealed a number of essentially adult teeth. Microscopic studies of these areas presented all stages of dental evolution from the simple nests of ameloblasts and characteristic adamantinous structures of the primitive enamel organ to the fully formed tricuspid structures, including enamel, dentine and pulp. The adamantinomas, described by Frazier and Alpers and others, differ fundamentally from this tumor in that no adult forms are reported in their cases beyond irregular and somewhat questionable enamel masses. The tumors in their series reputedly arise from the craniopharyngeal pouch, contain only

buccal elements and may be classified as epidermoids. This case, on the other hand, presents a multiplicity of structures derived from all three germ layers, both embryonal and adult in form. There are connective tissue elements varying from dense collagenous structure to the open framework of areolar tissue. Masses of hyaline cartilage are frequent, in some instances presenting early ossification, in others completely formed lamellar bone with Haversian systems. In other areas primitive membranous bone is noted. The many cysts and interconnecting spaces are lined by tall, columnar, ciliated epithelium, rich in goblet cells and forming numerous out-pouchings. These vary from simple to compound racemose seromucinous gland structures, constituting a picture remarkably like that of the nasal mucosa. In one area thyroid-like tissue is present. Widespread throughout the mass and forming a large portion thereof are well defined nerve tissue elements, both glial and ganglionic. Pineal tissue also is scattered through many areas, presenting the picture of embryonal undifferentiated structures, as well as the characteristic groupings seen in infancy. The fibrosed substance of the so-called involuted pineal body is not found.

This observation of pineal and nerve tissues in close association is of interest in view of the wide difference of opinion existing among different workers as to the presence or absence of nerve and glial elements in the pineal body.

Careful questioning of the family failed to reveal any psychical, sexual or developmental abnormalities. Past studies would indicate that adenomas of the pineal tend to retard sexual development and maturity (Marburg), and that teratomas induce sexual precocity and maturity (Askanazy). It is possible that in this case a balanced result of two antagonistic influences is manifest.

Discussion

(Dr. Harry C. Schmeisser, Memphis.) It may be of interest, in relation to this case, to bring to the attention of the members of the Association that I am exhibiting downstairs, among my photographs of clinical and anatomical lesions, a photograph of a brain showing a dermoid cyst in the fourth ventricle which was the cause of an internal hydrocephalus.

LIPOID HISTIOCYTOSIS WITH TUMOR-LIKE INVOLVEMENT OF SUBCUTANEOUS FATTY TISSUE, AND WITH DIABETES INSIPIDUS. K. L. Terplan and (by invitation) Roger S. Hubbard, Buffalo, N. Y.

Abstract. An exceptional case of lipoid histiocytosis was observed in a white woman, 34 years of age. This case differed from those reported as Christian-Schüller's syndrome in the presence of tumor-like infiltration of large areas of the subcutis, and in the absence of bone tissue changes and of exophthalmos. Marked polydipsia and polyuria had been present. The appearance of the chest clinically suggested a diagnosis of malignant invading blastoma or Hodgkin's disease. Deposits in the dura, with sinus thrombosis, small granulomatous structures in the serosa and somewhat similar structures in the lungs, pointed to a relation to Christian-Schüller's syndrome. Involvement of the bone was restricted chiefly to the bone marrow and was especially marked in long bones, less marked in ribs and vertebrae, producing marked anemia with signs of erythropoiesis, as in carcinomatous invasion of the marrow. Other important findings included thrombosis of the splenic vein with extensive older infarction, thrombosis of large iliac and pelvic veins extending into the inferior vena cava,

marked atrophy of thyroid (6.3 gm.) and moderate storage of lipoid in the mediastinum, retroperitoneal fat tissue and peripancreatic tissue. The mesenteric fat was not altered.

Histological analysis pointed to gradual replacement of the subcutaneous fat cells by huge foamy cells, stored with double-refracting lipoids, and subsequent reactive inflammatory changes with fibrosis. From stains with sudan III, Nile blue sulphate, and Weigert hematoxylin (L. Smith), most of these lipoids were cholesterol esters, particularly, according to Böhminghaus, cholesterol oleates. Histological study pointed in some areas to the appearance of huge cholesterol masses in the otherwise structurally normal fat cells. This deposition was especially plain in the fatty islets which were still preserved on the borders of large masses of foamy cells and granulation tissue. The endothelial cells in the liver, spleen and lymph nodes did not show storage of lipoid. The spleen showed changes in connection with venous thrombosis and its consequent hemorrhages. The pituitary showed practically complete fibrosis of the posterior lobe with some inflammatory cells, especially plasma cells. When the diagnosis of unusual storage of double-refracting lipoid in the subcutaneous tissue was made from biopsy it was already suggested that the case differed from those ordinarily attributed to a disturbance in cholesterol metabolism. Even though the lipoid infiltration of the subcutaneous tissue had markedly increased during the patient's stay in the hospital, at no time was the blood cholesterol increased. Atheromatosis was very slight. Lipoid content of adrenals was normal.

The aforementioned clinicopathological findings obviously assume a unique character when this case is compared with the usually described example of Christian-Schüller's syndrome. It must be mentioned that huge tumor-like nodules composed of cholesterol fatty acid esters were described by Proeschler and Meredith in the skin and abdominal cavity of a 32 year old woman under the heading "multiple myxocholestolipomata." However, this last case did not show cholesterol deposition in other organs, or diabetes insipidus.

In the dried tumor tissue 57.5 per cent was acetone-soluble lipoid. In this lipoid 57.4 per cent was cholesterol ester, apparently almost wholly cholesterol oleate, which was purified by crystallization from methyl alcohol and identified by analysis and a melting point determination. Other lipid constituents found in the material analyzed included free fatty acid (15.7 per cent), neutral fat (13.8 per cent), free cholesterol (3.9 per cent), and phospholipoid (2.6 per cent).

PSEUDOCANCER OF THE STOMACH. Anatole Kolodny, Sioux City, Iowa.

Abstract. An inflammatory mass was found in the stomachs of two patients. In both cases the clinical symptoms and the X-ray findings suggested a carcinoma of the stomach. At operation a wide resection of the stomach was done in each case. Grossly the lesion suggested a malignant growth, but the histology proved the lesion to be inflammatory. The patient who survived the operation is now well, nearly 3 years after the operation.

Discussion

(Dr. James Ewing, New York.) I am very much interested in this communication of Dr. Kolodny. I do not think, however, that he has given us sufficient histological evidence to determine whether he may still be dealing with one of those types of cancer of the stomach which have a tendency to show very

marked regression and atrophy of the cancer cells or not. I should like to have an opportunity to study his sections in detail.

(Dr. Kolodny.) I shall be very glad indeed to give the entire pathological material to anybody desiring to study it. I shall be very glad to have Dr. Ewing's opinion. I did not want to enlarge on the gross description of the specimen removed because of lack of time. However, the removed lymph nodes, which at first suggested malignant involvement, proved on final section to be purely inflammatory in nature; the normal portion of the stomach was greatly swollen, so that on the operating table it was difficult to determine where the tumor stopped. The matting together of the lesion with the pancreas, the duodenum and omentum was so widespread, much more so than one sees in carcinoma, that these findings, together with the histological appearance of the tumor, led us to believe that we were dealing with a benign lesion.

SPLEEN WITH ARTERIES ENCLOSED BY VEINS. Harry C. Schmeisser, Memphis, Tenn.

Abstract. The patient, C. L., 50 years old, a male negro, was admitted to the Memphis General Hospital, May 20, 1933, and died the following day.

The clinical and the autopsy findings had no bearing on the subject in question, except that an extensive examination of other arteries and veins grossly and microscopically showed no anomaly. There was also a moderate degree of generalized arteriosclerosis and a bilateral bronchopneumonia. To the latter his death was attributed.

The spleen was of average weight and size and of the usual external appearance. The splenic artery and vein outside of the spleen were normal. As the branches of each entered the hilum and ran in the trabeculae the artery became enclosed by its companion vein. This was clearly observed by serial section and dissection along the course of a particular pair of vessels, exposing them in long and cross-section. As the vessels approached the periphery of the spleen they separated on leaving the trabeculae.

Sections for microscopic study were taken of paired vessels and showed an artery and a large vein within a trabecula. The various layers of the wall of the artery were recognized and over a portion of its adventitial surface it was attached to an outer circular tissue which was either an arch of the vein and trabecula, or trabecula only. Here and there were endothelial-lined blood cells containing spaces, which suggested the remains of the vein lumen. Surrounding the remaining larger part of the adventitial circumference of the artery was the endothelial-lined lumen of the vein, which was continuous by probing and sight with the lumen of the splenic vein. In a similar manner it was proved that the microscopic arterial lumen was continuous with that of the splenic artery.

We were either dealing with a pseudo- or true enclosure of artery by vein. By pseudo-enclosure is meant that the artery running parallel to the vein in the trabecula had pressed into the vein to such a degree as to be almost entirely surrounded by the same and give the appearance in the gross that the artery was actually lying within the lumen of the vein. By true enclosure is meant that the artery had penetrated into the lumen of the vein. In this case it will be necessary to assume that the endothelium which lined the lumen of the vein had proliferated over most of the circumference of the adventitia of the artery, and had disappeared in places where the same was attached to the intima of the vein. A more detailed study will be made.

THE TYPES OF ARTERIOSCLEROSIS. Oskar Klotz, Toronto, Canada.

Abstract. The modern conception of the subject of human arteriosclerosis was reviewed with the aid of lantern slides. The author in the presentation of his subject made a plea for more careful observations at the autopsy table of arterial lesions, their nature and distribution. There is ample opportunity for all pathologists in participating in studies upon arteries, and there is still a variety of lesions in intima, media and adventitia which remain to be described and correlated with systemic disturbances. It is necessary, however, that all speak in the same "scientific tongue" and apply a given terminology with a definite and specific meaning. It was pointed out that Lobstein (1833), who coined the term arteriosclerosis, gave it a clear definition, from which we are not privileged to divert according to our own notions. So, too, the term atheroma has a definite meaning as indicated by Haller (1756). Atheroma is not synonymous with arteriosclerosis, nor does atheroma refer to any lesion containing fats and lipoids. The lesion lies in the intima, and consists of a cavity containing free fats, lipoids and cholesterol compounds in quantity sufficient to recognize a grumous mass. Fat in cells is not atheroma. Of late years the term atherosclerosis (Marchand 1904) has been badly misused. It is only necessary to observe the use of this word in a recent book on arteriosclerosis, and note how each of many authors adds confusion to our literature by his careless application of the term to realize that the word has lost its meaning. Two or three decades ago Virchow's term "chronic endarteritis" (1856) suffered similar mis-handling. It is evident that much misunderstanding by different investigators has arisen through improper use of definite terms. Investigators must be equally careful in their observations and in their manner of recording those observations.

The demonstration of the types of arteriosclerosis gave in review the important lesions found in all three coats of the arteries. The importance of the sclerosis in the arteries of the heart, kidney and brain was stressed, but it was also shown that the vascular lesions in these three organs are not identical, nor do they arise under similar conditions. The renal arteriosclerosis particularly has characteristics of its own, showing an inflammatory background and not developing the quality of an atherosclerosis. The progressive atrophy of the media with fibrosis, as seen in the cerebral vessels opposite to the nodular internal sclerosis, is distinctive for this group of vessels. Much careful analytical work must now be carried out to distinguish properly the qualitative differences in the several types of intimal sclerosis.

The discussion of arteriosclerosis must include lesions of the media. Pathologists are only awakening to the importance of the pathological processes which are found in this layer. Inflammatory processes, as found in rheumatic fever, sepsis, typhoid and other infections lead to scars which are later recognized as sclerosis, in association with intimal thickenings overlying them. In other cases the middle coat of the artery suffers degenerations of a mucoid, fatty or calcareous kind. The mode of origin of these separate processes is not known and offers opportunity for extended research.

Much investigation should be encouraged for a better knowledge of the lymphatic drainage of the arterial wall and the innervation of the arteries. A clearer understanding of the normal structure and function of the component tissues of the arteries would assist materially in interpreting the pathological events which take place in these vessels.

The author also reviewed the varieties of scleroses that had been induced in experimental animals. These lesions have been of great interest in indicating the manner in which they arise in animals, but care must be taken in applying these findings to the human. The hypercholesterolemia induced in rabbits leading to lipoid deposits in the arteries is a mechanism quite different from that related to similar lesions in man. A wide scope of studies awaits further investigation and calls for the combined interest of the biochemist and pathologist.

COMPARATIVE CHEMICAL AND HISTOLOGICAL EXAMINATIONS OF AORTAS FOR CALCIUM CONTENT. Samuel R. Haythorn and (by invitation) Fred A. Taylor, Helen Whitehill Crago and Anna Zoe Burrier, Pittsburgh, Pa.

Abstract. Parallel chemical and histological examinations were made on fifty-two aortas from patients of various ages, and the findings compared. The routine consisted in taking three samples from each aorta and in submitting them both to chemical analysis and to microscopic study by paraffin and frozen section methods. The samples were taken respectively from the ascending, thoracic and abdominal portions of each aorta. The chemical samples were taken from areas bordering on each side of the pieces for microscopic examination. When possible, exactly 10 gm. of aorta per sample were used. The aortas were stripped of adherent tissues, were submitted to the Corey-Denis method for calcium content, and reported in milligrams of metallic calcium per 100 gm. of wet aorta.

The results were grouped according to the ages of the patients in decade periods. In the groups under 40 years of age the chemical and histological findings were consistent, save for two aortas; one of these was from an infant with severe rickets and the other from a 38 year old patient with luetic aortitis. Thirty-two aortas came from patients over 40 years old. With each succeeding decade the comparative findings were more inconsistent and less easily interpreted. The average calcium content of 11 aortas in the 41 to 50 age group was 234.5 mg., of 10 aortas in the 51 to 60 group was 590 mg., and of 10 aortas in the 61 to 70 group was 553 mg. Two atheromatous aortas, both from 60 year old patients, explained the fact that the 51 to 60 group was higher than the 61 to 70 group.

An outstanding feature of the work was the great variation in the amounts of calcium found at different levels of the same aorta where there was a different degree of atheroma present in the given levels. Throughout all three of the older age groups the heaviest deposits were found in the segment above the bifurcation. The highest amount found was in an abdominal segment presenting numerous large calcium plates with ulcers about them and was 2312 mg. In a general way the calcium plates yielded the greatest amount of calcium, the combined cystic nodules with microscopically demonstrable medial calcium next, and those with medial calcification third, although there were none of these in our series that were not complicated by nodules. The amounts found in intimal cysts were so inconsistent that no conclusions were drawn.

None of the histological methods gave more than a general idea of what the chemical report was likely to be. Silver nitrate, while not considered specific, was perhaps the best indicator of total calcium, particularly where the test was made in bright sunlight. A modification of Cameron's alizarin stain yielded beautiful preparations, but failed to stain all of the calcium present.

THE RELATION OF CHOLESTEROL TO ATHEROSCLEROSIS. Timothy Leary, Boston, Mass.

Abstract.

(A) *Human Coronary Sclerosis*: Study of the lesions of coronary sclerosis and thrombosis demonstrates: (1) that atherosclerosis is a disease and not the inevitable consequence of age, since it occurs in early life and is frequently highly selective in its localization. (2) That the process is metabolic and not inflammatory. Such lymphoid cell infiltration as may be found in coronary lesions is on the whole infrequent, is accidental and not necessary, and is a late phenomenon when found. (3) That in the young human being progressive fibrosis is the usual picture in narrowing of the coronary lumen. Lipoid cells filled with cholesterol esters are deposited in the intima beneath the endothelium, but are replaced by young fibrous tissue before any considerable accumulation of these cells can occur. The deposit of the lipoid cells is progressive or cyclic, and a growth of young connective tissue replaces them *pari passu*, until the lumen is markedly narrowed. (4) That in the aged, large collections of lipoid cells arise with a minimal amount of fibrous tissue support. The delicate fibrous tissue (reticular) partitions between these cells are dependent upon a lymph or plasma diffusion, by imbibition through the endothelial layer from the blood in the arterial lumen. As the cell masses accumulate the nutrition of the cells tends to suffer and massive necrosis occurs, followed by liquefaction, producing so-called atheromatous "abscesses." (5) That the standard cause of death in young individuals is coronary thrombosis. This is due to necrosis arising in the sub-endothelial layer of the new fibrous tissue of the intima and extension of the necrosis to the endothelium. Apart from thrombosis, death in this group may be due to narrowing of the lumen and coronary insufficiency, under conditions of stress. The circulation through the narrowed vessels, while adequate under ordinary conditions, becomes inadequate under increased demands for blood. (6) That the standard cause of death in older individuals is the rupture of an atheromatous "abscess" into the coronary lumen, with secondary thrombosis. Rupture from the lumen into the atheromatous pockets also occurs. Death in this group may also be caused by coronary insufficiency (due to the narrowed lumen), under stress, without rupture of an atheromatous "abscess" or thrombosis. (7) Lesions of the elastica, media and adventitia are secondary, apart from possible stress effects. The primary process is lipoid, metabolic and intimal.

(B) *Human Coronary and Experimental Rabbit Atherosclerosis*: Comparative study of the lesions of human coronary and rabbit experimental atherosclerosis demonstrates: (1) that the lesions of human coronary sclerosis may be reproduced in rabbits by feeding cholesterol. Intimal sclerosis (atherosclerosis) occurs spontaneously in much less than 1 per cent of young rabbits. It was produced in 92.6 per cent (25 out of 27) of young rabbits in our series fed with cholesterol. (2) That in young rabbits, as is true in young human beings, intimal fibrosis in arteries is the standard lesion, atheromatous "abscess" formation the highly exceptional process. The fibrosis, *i.e.*, the reparative response, which arises in atherosclerosis in the arteries of the young is, therefore, a reaction of youth and not of species.

Any metabolic agent capable of causing atherosclerosis must have been a part of the diet of human beings from the earliest times, since atherosclerosis has been found in the bodies of mummies. We think of cholesterol in terms of blood cholesterol or bile cholesterol. This, however, is only the mobilized

cholesterol. Cholesterol is a substance required by every animal cell. Starling conceives of it as the stable substance of the animal cell — the framework — in the interstices of which the more labile substances undergo their metabolism. The greatest need for cholesterol arises in times of most rapid cell production. Egg yolk is intended for the embryo. Milk is intended for the infant. The increased blood cholesterol in pregnant women is mobilized to supply the fetus with this substance essential for its cells. Man is the only animal that ingests eggs and milk throughout its lifetime. Man is also the only animal, as far as is known, that dies in early life of coronary thrombosis and exhibits atherosclerosis almost universally with age.

The analogy of this metabolic disease to diabetes is close, even though cholesterol, in contrast to sugar, is a substance of difficult metabolism, and tends to accumulate within the body, *i.e.*, to be stored. As in diabetes, inheritance of a poor cholesterol metabolism results in manifestations of the disease in early life. As in diabetes, the advent of age, with the development of inefficiency of the cholesterol metabolism, is associated with more frequent late evidences of the disease.

Recent human experience in diabetes supports the experimental rabbit findings following cholesterol feeding. During and following the period when excessively fat diets were used in the treatment of diabetes, there was so great an increase of atherosclerosis that Shields Warren wondered whether the increase was due to diabetes or to the treatment of the disease. X-ray examination of the legs of children disclosed evidence of calcified atherosclerotic leg arteries, and xanthomas were common. Recent reports from the Joslin clinic, under a lower fat diet in the treatment of diabetics, indicate that xanthomas no longer appear, and X-ray evidence of calcified leg arteries in children is lacking.

HISTOPATHOLOGY OF THE CONDUCTING MECHANISM IN A HEART WITH VARIOUS DEGREES OF BLOCK DUE TO ARTERIOSCLEROSIS. Mortimer Cohen (by invitation), Pittsburgh, Pa.

Abstract. Degeneration of the conducting mechanism is associated with sclerosis of the arterioles supplying the system. The sclerosis involves the intima primarily with definite encroachment upon the lumen. This interference with the blood supply results in shrinkage of the component parts of the system. The extent of the shrinkage, however, is not the same over the entire mechanism. In the sino-auricular node the involvement is moderate; it is greater in the auriculoventricular node and in the main bundle. The left branch is reduced to a few fibers. The shrinkage is due to actual disappearance of some individual fibers and distinct narrowing of the surviving fibers.

The connective tissue is increased in the mechanism itself, but the greatest increase is seen in the fibrous portion of the interventricular system.

The electrocardiogram demonstrates various degrees of block varying from latent to complete. This variation may depend on the ability or failure of the surviving damaged fibers to transmit impulses.

Sclerosis of the arterioles is present elsewhere in the heart with degeneration and replacement fibrosis. This damage is pronounced in the interventricular septum. Here the thebesian veins are distended, apparently attempting to supply the injured area with blood.

Discussion of Papers by Drs. Klotz, Haythorn and co-authors, Leary, and Cohen

(Dr. Harry C. Schmeisser, Memphis.) In relation to Dr. Cohen's paper, permit me to call attention to a very interesting case of heart block caused by gumma of the septum, published by Bridgman and myself in the Johns Hopkins Hospital Reports, 1916, in which the complete clinical and pathological findings could be compared. The lesion, in this case, was readily seen with the naked eye, measuring 4.5 by 3 by 1.5 cm., and histologically was found to involve the bundle of His. Heart block due to arteriosclerosis, as presented by Dr. Cohen, required a more detailed histopathological study.

(Dr. Howard T. Karsner, Cleveland.) Many points of interest have been drawn to our attention by the excellent studies presented this afternoon. In order to initiate further discussion, there are a few items to which I wish to refer. As Secretary I take this opportunity to express publicly to Dr. Klotz the warm thanks of the Council for the clarity and inclusiveness of his paper, the summary of many years of intensive study of arteriosclerosis. Of great interest is Dr. Leary's material, carefully studied and beautifully presented. In regard to his work I wish to express the view that the influence of syphilis on coronary arteries cannot be dismissed, especially when as the result of Moritz's studies of 8 cases of syphilitic aortitis a lesion of coronary arteries, which occurred within the first 2 or 2.5 cm. from the origin, was identified as syphilitic in all the cases examined (*Arch. Path.*, 1931, 11, 44). It is undoubtedly true, as Dr. Leary points out, that there must be significance to the lipoid deposits in the development of coronary and other forms of arteriosclerosis, but whether this represents cause or effect is at the present time a matter of interpretation. I have studied carefully the coronaries in more than 50 rheumatic hearts and am impressed by the constancy of significant lesions of these vessels (*Am. Heart J.*, in press). I do not mean to imply that these lesions are specifically rheumatic, but regard them as manifestations of infectious disease. Reference has been made to the Macy Foundation Survey of Arteriosclerosis, edited by Cowdry. In this volume, McCallum expresses the view that there is little ground for regarding infectious diseases as of importance in the production of arteriosclerosis. As a result of my studies of rheumatic hearts, the studies of Klotz and of many others, I think that consideration must still be given to the significance of infectious diseases in the development of arteriosclerosis in general and coronary sclerosis in particular.

Dr. Cohen's studies clearly indicate the importance of arterial disease as a cause of heart block. Although in the earlier case reports gumma was emphasized, it now is apparent that coronary sclerosis is a considerably more frequent cause of heart block than is gumma. Dr. Cohen's demonstration of the communication between arterial channels and the thebesian system is striking. While I do not wish to state that thebesian circulation is not of importance in maintaining myocardial nutrition, nevertheless, the rediscovery by Moritz and his associates of Langer's observations showing anastomosis between coronary arteries and vessels in pericardium and mediastinum must be considered (*J. Exper. Med.*, 1931, 56, 919, 927). It is at least possible that these extra-cardiac anastomoses of the coronaries may play a part in nutrition of the heart muscle when intracardiac coronaries are reduced in lumen or occluded.

(Major V. H. Cornell, Washington.) There is one thing I should like to ask Dr. Cohen, and that is if the hearts were studied in serial sections. I ask this

because I have recently been working with Dr. Yater on several such cases (we have a series at Washington). In his summary of the literature he states that many cases must be discarded because complete material has not been studied. Perhaps personally I did not appreciate that as fully until a few days before I left Washington when we saw a case without apparent severe coronary involvement, but with calcification of the mitral valve at the base of the aorta with a spur jutting out just beneath the mitral leaflet. On serial section after running through several trays the bundle showed a small spot of calcium at the center. This rapidly grew to the full size of the bundle with complete obliteration of the bundle and then disappeared in 170 serial sections, which approximated 1.5 mm. of the bundle. You can see how readily such a lesion would be missed if serial section was not used.

Another point which occurred to me was that although in the case presented we see so much sclerosis of the coronary branch, we do see a compensatory circulation, and the question arises as to whether the lesion of the bundle is due to circulatory deficiency, or whether it is not a part of the general fibrosis occurring in all the tissue around it, and whether that fibrosis itself is primarily due to circulatory disturbance or to some other factor.

(Dr. E. T. Bell, Minneapolis.) There is one form of arterial disease which has not received as much attention as it deserves, I think, and that is fibrosis of the media. If you study the media of the muscular arteries you find in young persons there are just a few collagenous fibers between the muscle cells. In middle age, in the forties, there is a decided increase in the collagenous fibers, and in advanced age, seventy to eighty, the muscular arteries of the extremities, particularly, are almost completely replaced by collagen, so that commonly there are no muscular fibers left. That is a very striking alteration and can be brought out beautifully with the azocarmine stain, and is not related to any factor, so far as we know at present, except age. One is impressed in studying arterial disease with the variations of the anatomical type in different arteries. The changes in the muscular arteries are quite different from those in the elastic arteries. In the muscular arteries the changes are chiefly a medial fibrosis, a medial calcification, and in the elastic arteries the outstanding changes are intimal. The most prominent feature of arterial disease, what we call arteriosclerosis, is age, and that seems to be much more important than anything else. That there are other things is brought out by the fact that we frequently have arterial disease, even fatal types, in young persons, which encourages us in the search for the factors that will influence it. We must not forget that one thing which influences intimal disease is diabetes. If you have had much experience in postmortem examinations you know that intimal disease is usually much more prominent in diabetics than in non-diabetics of the same age, and disordered metabolism is therefore one possible etiological factor.

(Dr. N. C. Foot, New York.) It might be well to point out in connection with animal experimentation along these lines the importance of knowing the genetic history of the rabbits with which you are working. Out in Cincinnati, Zeek in our Department attempted to reproduce the Newberg experiments on a number of Belgian rabbits without any result, except that the renal lesions were reproduced. The aortas remained unaffected. Later on the rabbits in our hutch were found to develop arteriosclerosis spontaneously, and Zeek found that one buck in particular seemed to possess the faculty of producing progeny, all of which developed spontaneous arteriosclerosis; so that, had we used that particular strain to work with, we would undoubtedly have produced very nice arterio-

sclerotic lesions, which, had we not known the history of the animals, might have been explained to our satisfaction on totally different grounds and had nothing to do with the experimental work in hand. Therefore, it is very important to know all about your rabbits' family histories before you begin on cholesterol feeding experiments, Newberg experiments, and the like.

(Dr. Leary.) First, with reference to syphilis of the coronary, I have examined a large number of cases of syphilitic aortitis and I have yet to see an example of syphilitic disease of the proximal portions of the coronaries, that is, of the portion of the vessels which lie beneath the epicardium. Even Warthin, who found syphilitic focal lesions universally, admitted that syphilitic lesions of the main coronary arteries were rare.

With reference to rheumatic lesions of the coronaries, it is possible that they may have a primary influence in some cases. The standard lesion of coronary sclerosis, *i.e.*, atherosclerosis, can and does appear usually independently of infection as such. Infection may lead to coronary stresses, which in turn may favor the deposit of cholesterol, but the absence of evidence of inflammatory reaction in early atherosclerotic processes negatives the claim that infection is a necessary or a common factor.

Dr. Bell said that the outstanding lesions in the muscular arteries were fibrosis of the media. That is not my experience with relation to the coronaries. Secondly, he brought up the relation of atherosclerosis to diabetes. I think the factor of diet in the experimentation which was carried out in the treatment of diabetes supports completely the rabbit experimentation we have been carrying on. You will remember that excessively fat diets were used some years ago, which led to such an increase in arteriosclerosis that Dr. Warren wondered whether the increase was due to diabetes, or to the treatment of the disease. Since the high fat diets have been discontinued, atherosclerosis is not so outstanding, the xanthomas which were common disappeared, and it is impossible now in the same clinic to get X-ray evidence of children's arteries which have calcified.

With reference to Dr. Foot's remarks, proper controls take care of that situation. Our controls were all negative, save that one rabbit showed two minute foci of medial sclerosis in the aorta.

(Dr. Bell.) The muscular arteries I had reference to are those in the extremities and abdominal viscera. It does not apply to the coronaries.

(Dr. Klotz.) I wish to thank you for the very kind reception you have given to the presentation of my paper. I also wish to extend my congratulations to Dr. Leary for the demonstration of a beautiful series of slides of coronary arteriosclerosis. Dr. Leary has clearly brought to our attention the frequency with which severe coronary scleroses are encountered in the relatively young as well as old individuals. The lesions which are encountered in the fatal cases are very similar in character, showing stenosis of the lumen of the vessel and marked lipoid degeneration of the intimal lesion. It is, however, difficult to determine the antecedent history of these human lesions at such a late stage in their development. Dr. Leary accentuates the importance of the finding of fatty materials in these scleroses. I am sure, however, that Dr. Leary does not wish to convey the idea that all coronary lesions arise in this manner, and that he will admit the development of others on an inflammatory basis. Such a one I illustrated in a child of 9 years, who after recurrent attacks of tonsillitis developed a glomerulonephritis along with a well marked coronary sclerosis. Another case showed similar endarteritis without lipoid changes in a girl of 14, who

had suffered a succession of severe infectious diseases during 2 years preceding death. It is very clear that our studies in the future must lay more stress upon the arterial lesions at their commencement rather than attempt to indicate the course of the lesions after they have attained their late characteristics after middle life.

I am sorry that Dr. Bell has suggested to us that age is a cause of arteriosclerosis. It is as though age were a crime. I am sure he did not mean that. It is true that with advancing years we accumulate the scars of previous damage and thus for the demonstration of numerous and well marked examples of arteriosclerosis we seek the tissues of elderly individuals coming to autopsy. Age, however, has not been a factor in producing these lesions. The wear and tear of life upon tissues is not so important as other essential factors which have a specific influence upon certain structures. It is, however, pleasant to think that there is no truth in the old belief that alcohol produces arteriosclerosis or that smoking brings about lesions in the human aorta. The experimental evidence that nicotine acts upon the aorta of some of the lower animals cannot be applied to the human subject. The manner of these experiments is entirely different from anything that happens to man.

Dr. Foot has brought up a very important point respecting the use of experimental animals in the study of arteriosclerosis. Nearly every one of the lower animals that is used for experimental purposes develops its own type of arteriosclerosis, and one must be very careful in distinguishing these lesions from such that may be induced by the experiments. On the other hand, too, as has been stated by Dr. Foot, some of these animals develop arteriosclerosis with great ease and at periods in their life when not expected. Some strains of animals show sclerosis in greater frequency than others belonging to the same species. Furthermore, in the study of arteriosclerosis the rabbit has been utilized most commonly for experimental purposes. This herbivorous animal is so different from the human that the greatest care must be taken in interpreting the experimental results. I am far from satisfied that the animal experiments, indicating cholesterol compounds as important factors in the production of arteriosclerosis, can be accepted for the interpretation of the human lesions. The cholesterol metabolism in the rabbit and in the human are two entirely different processes. It is my belief that the cholesterol-lipoid substances are only an index of the damage imposed upon the arteries, and that they do not represent the factors causing the lesion. In the future it may be well to turn from the rabbit and carry on experiments in the monkey or in other omnivorous mammals. In each case it will be necessary to familiarize oneself with the natural diseases arising in these animals as well as with the normal metabolism concerning various substances. The experimental work which has heretofore been carried out on a few of the lower animals has been interesting and has assisted in understanding reactions in the arterial wall. The experiments, however, are still inconclusive in respect to an appreciation of the human disease.

CARDIOVASCULAR RENAL CHANGES ASSOCIATED WITH BASOPHILIC ADENOMA OF THE ANTERIOR LOBE OF THE PITUITARY. H. E. MacMahon, Boston, Mass.

Abstract. In unravelling the rather complex clinical syndrome of pituitary basophilism one finds recurring with remarkable constancy a group of signs and symptoms indicative of cardiovascular renal disease; a greatly elevated blood

pressure, an enlarged heart, associated with frequent headaches and disturbances in vision. These may be present over a period of months or years, to be complicated at a later period by signs of renal decompensation, namely, inability to dilute and concentrate fluids, and a progressive retention of non-protein nitrogen substances in the blood stream. The urine will reveal albumin, casts of various types and red blood cells.

A study of such cases both clinically and from the standpoint of pathological histology has shown that this cardiovascular renal disease simulates that described by Volhard and Fahr as malignant nephrosclerosis.

Discussion

(Dr. Howard T. Karsner, Cleveland.) Will Dr. MacMahon state whether or not the acute necrotizing arteriolitis is present in all the cases of basophilic adenoma of the anterior lobe of the pituitary that he studied?

(Dr. MacMahon.) Acute necrotizing arteriolitis has not been a constant feature. I have seen it in 3 cases of basophilic adenoma of the anterior lobe of the pituitary. These patients showed signs and symptoms of advanced pituitary basophilism (Cushing's syndrome), including marked hypertension, renal decompensation of varying degree with red blood cells and albumin in the urine. Two days ago I received some kidney sections from Dr. Hussey, from a patient of Dr. Cushing showing signs of pituitary basophilism. Clinically there were no signs of renal decompensation and the urine contained no blood. Histologically the only remarkable lesion in the kidney was a vascular hypertrophy and marked swelling of the basement membrane of the small arterioles.

(Dr. Kornel L. Terplan, Buffalo.) The relation of the basophilic cells in the hypophysis to hypertensive disease is of great interest, although we have not arrived as yet at a clear conception of the etiological rôle of the basophilic cells or basophilic adenomas in this connection. From the papers of Kraus, Berblinger and Zeynek we know that the number of basophilic cells is considerably increased in different diseases, especially in those with a high cholesterol level, such as marked essential hypertension and chronic glomerulonephritis. This also has been observed in cases of severe obesity, regardless of whether or not they showed hypertension. In 1 case of basophilic adenoma of the hypophysis, which I saw in Prague, the only one I recall, there was no associated hypertension.

(Dr. MacMahon.) I think it is quite possible that one may find a basophilic adenoma of the pituitary in the absence of vascular renal changes. It is quite true that one finds other examples of adenoma appearing in glands of internal secretion without showing any clinical manifestations. In the case of the basophilic adenoma of the anterior lobe of the pituitary showing signs of pituitary basophilism, the cardiovascular renal signs and symptoms may in the early stages be slight or absent; whereas a careful study of the advanced cases may throw some light on our problems of cardiovascular pathology. Several months ago Dr. Russell, of the London Hospital, England, told me of a case of basophilic adenoma of the anterior lobe of the pituitary showing the histological changes in the kidney characteristic of malignant nephrosclerosis. It does seem more than a mere coincidence that this relatively rare vascular lesion should recur so often in the advanced cases of pituitary basophilism.

PULMONARY EMBOLISM FOLLOWING TRAUMA. J. S. McCartney, Minneapolis, Minn.

Abstract. A study of the protocols of 9882 postmortem examinations done during 6½ years reveals that in 1604 instances, or 16.2 per cent, death followed injury. In the traumatic group embolisms were found in 61 cases (3.8 per cent), and in the non-traumatic group in 222 cases (2.6 per cent). Statistically this appears to be a significant difference. The age distribution of cases was the same in the two groups. There were 6283 males and 3598 females. The incidence of embolism was 2.4 per cent in the males and 3.5 per cent in the females. There is a significant difference in the two sexes in the incidence of embolism. The greater incidence in females is not to be accounted for by the embolisms that followed childbirth.

The incidence of embolism after trauma was 2.6 per cent in the male, and 7.7 per cent in the female. This is a significant difference.

Of the 61 traumatic cases 45, or 73.7 per cent, were between the ages of 50 and 79 years. In the non-traumatic group but 59 per cent occurred between these ages.

The incidence of embolism after skull fracture was 0.2 per cent, after fracture of the ribs 0.9 per cent, after fracture of the spine 1.4 per cent, after fracture of the upper extremity 4.5 per cent, after fracture of the pelvis 3.2 per cent, after multiple fractures 4.8 per cent, after fracture of tibia and (or) fibula 14.8 per cent, and after fracture or dislocation of the femur 25 per cent. In the group of miscellaneous traumas there was an incidence of 2.2 per cent. No embolisms followed gunshot wounds. These percentages indicate the great importance of rest in the etiology of embolism, since fractures of the lower extremity require much more prolonged immobilization than other fractures. The part which operative procedures played in this series is indeterminate, although 14 were operated upon for the trauma sustained.

Before 50 years of age the maximum incidence, according to the number of cases, was only 2.9 per cent. Between 50 and 79 years of age the incidence was 7.2 to 7.3 per cent, and from 80 to 89 years of age 4.8 per cent. The very great importance of age in the causation of embolism is clearly demonstrated.

Of the 61 embolisms, 53 followed fractures or dislocation and of these 53 embolisms 28 were fractures or dislocations of the femur and 7 fractures of the tibia and (or) fibula. Only 4 of the 53 fractures were compound.

The times of occurrence of pulmonary embolism after trauma were: 1st week 9 cases, 2nd week 21, 3rd week 10, 4th week 5, and after the 4th week 16. Embolism following trauma takes place later than after operation.

Discussion

(Major V. H. Cornell, Washington.) I should like to ask if you were using a frame for the suspension of the lower extremities in the treatment of fracture, or was the leg completely immobilized?

(Dr. Thomas H. Belt, Toronto.) Dr. McCartney's interesting paper touches upon a subject to which we have been devoting considerable attention here in Toronto, namely, the autopsy incidence of pulmonary embolism. It brings out the striking frequency of embolism following trauma. We have found here, since we have made a special search for emboli in the lungs, that they occur in a large number of cases that ordinarily might be passed over if the search was

not carefully carried out. I refer particularly to the smaller types of emboli that cause infarcts of the lung, and which I believe Dr. McCartney has excluded from his series. We find that about 10 per cent of our routine autopsies show emboli that are demonstrable to the naked eye, and a large proportion of these cases have cardiac incompetence; many of them actually died from heart disease. There are many factors in the causation of thrombosis and embolism which we have to consider, and the most important etiological factor in our series seems to be slowing of the circulation.

(Dr. McCartney.) In response to Major Cornell, I cannot answer the question as to immobilization. I have not studied as yet the hospital records from that standpoint.

I might add in connection with what Dr. Belt had to say, that, in the non-traumatic cases, cardiac lesions of one type or another stand out prominently, but this is not so true in the traumatic cases.

ANATOMICAL AND EXPERIMENTAL OBSERVATIONS ON AIR EMBOLISM. W. H. Chase (by invitation), Montreal, Canada.

Abstract. The autopsy findings are described in a case of air embolism which was rapidly fatal following the incision of a large branch of the right pulmonary vein during a lobectomy operation. On opening the cranial cavity first there was found a diffuse subarachnoid hemorrhage over the tributaries of the longitudinal sinus and extending around penetrating cortical veins. Intracranial air emboli were confined to branches of middle and anterior cerebral arteries. The left heart and coronary arteries were also filled with air, but there were no perivascular hemorrhages.

In order to obtain a visual conception of living events in air embolism measured quantities of room air were injected into the aortic arch in a series of 12 rabbits, through a long cannula inserted through an opening in the right carotid artery. The observations were made possible by the adoption of an apparatus devised by Ricker and his co-workers for the study of the rabbit's mesentery *in vivo*, while it was bathed in Ringer's fluid at a constant temperature. This apparatus was particularly applicable because (1) tissue movements could constantly be observed microscopically through a water immersion lens with direct illumination, and (2), with practice, the operator could perfect a technique that permitted him to start with a vascular mesenteric bed that was almost a physiological preparation.

The experimental observations may be summarized as representing two distinct vascular effects. The first is traumatic and probably neurovascular, and the second is mechanical. (1) The traumatic neurovascular effect is independent of the nature of the irritant. It produces an immediate transient vasoconstriction of muscular arteries and terminal segments, during which the slow peripheral blood movement (prestatic state) is associated with slight diapedesis. As fatigue of muscular arteries translates vasoconstriction to vasodilatation there develops a progressive arterial hyperemia. (2) The mechanical vascular effect is due to the specific agent which is the air obstructing small arteries and arterioles. It also produces peripheral prestasis which is of rapid onset and sometimes prolonged. During this interval there occurs a more or less abundant diapedesis of red cells from the capillaries and venous side of terminal segments. This ends in either complete cessation of blood movement (stasis) or in diffusion of air through the arterioles with a return of the hyperemic state.

In both the autopsied case and in the animal experiments air emboli were confined to arteries and arterioles, while hemorrhages were confined to terminal vascular segments and small veins. Although we must accept with caution a comparison of tissue movements in animal experiments to human pathological processes, the similarity of the vascular changes in the rabbit's mesentery to those in the meningeal vessels of the autopsied case, under stimulus of the same irritant, suggests a similar neurovascular mechanism concerned in their production.

Discussion

(Dr. Esmond R. Long, Philadelphia.) I am wondering whether Dr. Chase has given some thought to that very distressing accident which occurs now and then, sudden death during the course of artificial pneumothorax. As you know, sometimes in the course of the therapeutic administration of air in a pleural cavity the patient goes into a state of collapse and dies within a few minutes. There have been two theories as to the cause of death. One is that death is due to introduction of the needle into the pulmonary vein with resultant direct air embolism. This theory has not been substantiated by postmortem examinations. In its failure a second theory has been accepted by a good many clinicians, the rather nebulous hypothesis of pleural shock. In the first case reported by Dr. Chase it is possible that a vein was punctured. If so, after the needle was withdrawn, could a sufficient amount of air have entered the pulmonary vein from the pleural cavity to cause embolism? Sometimes death occurs after 200 to 300 cc. have been put in the cavity. There is no question in these cases that during the administration of air the needle is not in the vein; the manometer shows it to be in the pleural cavity.

(Dr. Timothy Leary, Boston.) Medicolegal deaths from air embolism are rather rare, but sudden death is always due to a massive introduction of air into the circulation, making a mixture that cannot be pumped. One finds the right side of the heart filled with this frothy blood constantly in my experience. I have seen at least 10 or 15 of these cases. I have performed autopsies upon 2 cases of sudden death from pleural shock following the introduction of a needle into the lung for the purpose of obtaining lung cultures in pneumonia. There was no possibility of the introduction of air in these cases.

(Dr. Long.) I am glad to hear Dr. Leary's comment, because in Chicago we always taught that death in air embolism could occur only in the way he describes. However, it is commonly held by clinicians who do pneumothorax work that fatal embolism can take place from small amounts of air on the other side of the vascular circulation.

(Dr. Edwin F. Hirsch, Chicago.) I should like to ask Dr. Chase whether he considers that the experiments done on the peripheral circulation apply to the pulmonary circulation. There has been doubt that air introduced into the pulmonary artery passed through the capillary bed of the lungs and entered the systemic circulation.

(Dr. Marcus W. Lyon, South Bend.) I should like to cite one instance of accidental administration of air into the venous circulation. In an attempt to withdraw blood from a patient for immunization against influenza in 1918-19 the patient complained as the pump was being worked that he could feel something trickling up his arm, and the person operating it discovered that the pump was set in the wrong position, and instead of withdrawing blood, air was being

forced into the patient. Nothing happened, but the patient was sent back to the ward without further attempt at withdrawal of blood. Probably not much air entered, but certainly some.

(Dr. Harry C. Schmeisser, Memphis.) Dr. Chase's observations are very important in answer to the question so frequently put to the pathologist by the surgeon, whether or not air introduced into the circulation at operation could have a bad effect upon the patient and possibly be the cause of sudden death. In past years Dr. Welch taught that no bad effects would be experienced from air emboli except possibly, on operating about the neck, the internal jugular might be cut and a large amount of air pass to the pulmonary artery.

(Dr. Chase.) I think there is no doubt that it takes large quantities of air in the circulation to cause death in humans. We have had several cases of death from pleural shock, in none of which have we been able to demonstrate definitely the presence of air embolism. Several such cases have been described in the literature.

In regard to the passage of air through the pulmonary circulation, that does occur in some animals. It occurs in dogs, but there is nothing to indicate that it occurs in rabbits or in humans.

STUDIES ON THE DIGITAL VASCULAR SYSTEM WITH CONSIDERATION OF THE
CONDITION OF THE GLOMUS IN INFLAMMATION, ARTERIOSCLEROTIC GANGRENE,
DIABETIC GANGRENE AND THROMBOANGELITIS OBLITERANS. N. W.
Popoff, Rochester, N. Y.

Abstract. A brief summary of the work done on the human digital vascular system is presented. A digital glomus similar to glomus coccygeum is described. Anatomical data enabling understanding of heat regulation and other functions of the digital glomus are given. The importance of taking into consideration the state of the glomus for proper understanding of capillary reading, of local clinical manifestations and of various diagnostic tests is stressed. New histological points in differentiating arteriosclerotic and diabetic gangrene of the extremities are presented. A hitherto unknown vascular anomaly manifested by the presence of peripheral digital arteriovenous anastomoses is described and is offered as an etiological factor of thromboangitis obliterans.

THE AMERICAN JOURNAL OF PATHOLOGY

VOLUME X

NOVEMBER, 1934

NUMBER 6

THE FUNCTIONAL REACTIONS OF THE HUMAN THYROID *

A CONTRIBUTION TO ITS HISTOPHYSIOLOGY

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Many attempts have been made to get a better understanding of the histophysiology of the thyroid gland. If Graves' disease is nothing more than a severe condition of hyperthyroidism the structure of the enlarged gland should give us the key to the problem. Unfortunately the arguments in favor of a condition of dysthyroidism cannot be discarded altogether. On the other hand, the histology of toxic goiters (exophthalmic and non-exophthalmic) is by no means a constant one and the efforts made thus far to explain the severity of the clinical course by the histological features have not met with uniform success. The statistics of Wilson¹ published in 1914 show that the clinician and the pathologist are in agreement in only 75 per cent of the cases examined.

The use of the tinctorial reactions of the colloid, suggested by Kraus² and Troell,³ has not improved this percentage by any great degree. However, the concept of the "Wucherungspolstern" introduced by Sanderson-Damberg⁴ has enabled Hellwig⁵ to get more satisfactory results. This tends to prove that a better knowledge of the finer histological structures will clear up many questions of interest in the toxic goiter problem. Unfortunately, researches along these lines are now hampered with new difficulties which arise from the prevailing Plummer preoperative treatment with iodine (Mayer and Fürstenheim⁶). The accumulation of colloid is a disturbing factor in the interpretation of the morphological findings.

* Received for publication June 8, 1934.

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Another method of approach would consist in looking for distinctive morphological peculiarities of endemic goiters with or without hyperthyroidism. Here again a uniform morphological standard is lacking. Nothing enables us to say whether an enlarged gland does or does not elaborate more thyroxin. As Wegelin ⁷ points out, our shortcomings may be due to several causes, among which our imperfect knowledge of the histophysiology of the thyroid is a prominent one.

Progress in this direction is needed and the conditions are too intricate to make headway by the study of goiter material. In our opinion progress will be attained only by a scheme of investigation wherein the cellular reactions are closely correlated with a definite functional phase of development, such as metamorphosis (Uhlenhuth ⁸), or with a stimulation of the gland measured by the increase of the basal metabolism. The recent work of Okkels, Krogh and Lindberg,⁹ who make use of the thyrotropic hormone as a stimulant, opens no doubt a promising field of investigation. Technical difficulties, however, are met with which can be mastered only by a team of workers trained for this particular purpose.

We want to prove in this paper that, in the meantime, useful conclusions can be drawn from a systematic histological and cytological survey of an extensive series of human thyroids collected in the postmortem room. Such material must be fresh, perfectly fixed and should be collected in an area such as Ghent, Belgium, where endemic goiter is very rare.

Investigations of this kind have been carried out in recent years by Farrant ¹⁰ and Williamson and Pearse,¹¹ but their aim was different from ours. For the present at least we are not concerned with knowing whether, in one particular disease, the gland is hyperplastic or not, or whether the gland has a larger or a smaller hormonal output. We use marked changes brought about by pathological conditions to get new information about the functional interpretation of certain histological structures.

It is well known that infectious or toxic conditions sometimes considerably alter the thyroid morphology. What is more often overlooked is the fact that structures appear which are considered by many as a characteristic feature of toxic goiter. The extensive material at our disposal gives us the opportunity to link up a complete series of transitional changes, the study of which leads

to a satisfactory explanation of the extreme stages. We are thus able to connect the morphology with certain aspects of functional activity.

THE MORPHOLOGY OF THE THYROID EPITHELIUM DURING FOLLICULAR DEPLETION (COLLOID RELEASE)

The first point we shall consider is the morphology of the thyroid epithelium during follicular depletion. The work of our predecessors, as well as our own investigations, shows that colloid release is a common occurrence in toxic or infectious processes, but it is by no means constant. In 6 cases of pneumococcus lobar pneumonia we never saw any sign of it. Neither did we notice any depletion in acute streptococcal puerperal septicemia. In acute peritonitis following appendicitis, on the other hand, we found regularly a certain degree of colloid release. In patients dying from intestinal obstruction (11 cases) all the gradations of the process exist, so that finally the follicles are found to be reduced to canalicular formations which resemble in all respects similar structures seen in toxic goiters. The same remark applies to diphtheria cases, although in these there is no general rule, intercurrent infection probably accentuating the depletion.

Figures 1 and 2 represent the thyroid of a female, 54 years old, who died within 48 hours after the onset of intestinal obstruction; Figure 2 particularly shows the early stages of the depletion. Many follicles, however, are still filled to a maximum, as in Figure 1. The morphology of the epithelium offers many striking features. First of all, the epithelial lining is heterogeneous, contrary to what is generally stated in standard textbooks. To our knowledge only Aschoff¹² has laid stress on this fact. A narrow segment of high cylindrical cells with dark hyperchromatic nuclei is very evident, while the remaining epithelium is either low cuboidal or endothelioid. Thus we already can distinguish three types of epithelium in one follicle, low cuboidal (Type 1), columnar (Type 2), endothelioid (Type 4). Let us now examine the follicle on the left of Figure 2 and neglect for a moment the typical "Sanderson Polster." The high cylindrical segment is slightly extended. It is doubtful if in this follicle colloid release has begun. We should like to draw attention to the appearance of another type of cell, especially in the

groove adjacent to the "Polster." It is a high cuboidal cell with a large vesicular nucleus (Type 3). A general survey of 500 human thyroids from normal and pathological cases proves that all the varied aspects of the thyroid epithelium can be classified under these four types.

The follicle on the right of Figure 2 shows an unquestionable depletion. Its shape tends to be more or less triangular, while the high cylindrical epithelium extends over two-thirds of the contour.

In Figure 3 again we notice the close connection between the extension of this type of epithelium and the progressive collapse of the follicle. On the left the shape of the two adjoining follicles A and B proves that they are completely filled with colloid. A high cylindrical segment is easily recognizable in both, but it is narrow. On the right of this figure (follicles C and D) these high cylindrical segments extend over the greater part of the follicular wall, while the colloid depletion is already marked. This is still more convincingly emphasized in Figure 4 from another case of intestinal obstruction. Here the follicle is completely surrounded by high palisade epithelium of which the morphology is clearly demonstrated on higher magnification. We need not dwell upon its histological characteristics, except to point out the fact that the width of the cell does not far exceed the width of the oval, hyperchromatic nucleus.

We suggest that there is a close connection between the increase in the size of the segments of cylindrical epithelium and colloid depletion. Since we believe that this point is of importance in thyroid histology, we should like to present further data in favor of this assumption. Figures 5, 7 and 8 represent thyroids from diphtheria cases. Of the four large follicles present in Figure 5 the third from the left shows a beginning stage of depletion. Here the high cylindrical epithelium forms the least extensive segment. In the second and fourth follicles from the left, collapse of the follicle is much more marked. In the one where it is most evident (the fourth), the greatest part of the lining is columnar. Figures 7 and 8 again show this striking correlation; the generalized high cylindrical character of the follicular epithelium corresponds to extreme degrees of colloid absorption. Stress is laid upon the fact that in the human thyroid a large spherical follicle is never found with an entirely uniform, high columnar lining. When this type of epithelium surrounds the follicle entirely, the latter has always attained an extreme degree of de-

pletion. Of course, we find in our preparations sections of collapsed follicles without columnar epithelium. Serial sections prove, however, that they correspond to the tail end of a diverticulum of a depleted follicle, the main body of which, not seen in this particular section, is provided with large high cylindrical segments. Others are depleted follicles of which the epithelium shows regressive changes; they are devoid of functional activity. We may summarize our conception as follows: in a follicle provided with uniform and generalized high cylindrical epithelium, the colloid resorption is always considerable. As shown above the reverse is not true, *i.e.*, a depleted follicle may be found occasionally without cylindrical lining.

The above observations force upon us the conclusion that the columnar epithelium absorbs the colloid stored in the follicle and excretes the active hormone into the blood or lymph vessels. We see some cytological evidence of this excretion in the form of colorless basal vacuoles found only in this type of epithelium, as one of us¹³ has shown in a recent paper. They attain a considerable size in some cases of diphtheria, as shown in Figure 7.

Moreover, there is always a very marked vasodilatation of the sinusoids adjoining this type of epithelium. The lymph vessels are also distended; in cases of acute depletion this may lead to edema of the stroma (Fig. 7). The excretory function of high columnar epithelium is in itself not surprising since toxic goiters are so amply provided with it. A study of the latter would have led to this conclusion were it not for some exceptions which resulted in confusion. In the light of what follows, these discrepancies, and especially the eventual absence of high epithelium in a toxic goiter, can be easily explained.

THE EFFECT OF COLLOID DEPLETION

The result of colloid release on the architecture of the follicle is illustrated by Figure 7. The collapse of the wall leads to the formation of diverticula which later on sever all connection with the main follicle. In this way accessory minute follicles are formed which, in some instances, as in Figure 8, surround the depleted follicle as satellites. These diverticula of the main follicle have been noticed since Virchow by many authors (Wegelin,⁷ Marine,¹⁴ Wilson,¹⁵ Norris,¹⁶ and Rienhoff¹⁷). Most of these workers never took into account the part the disease played in the extension of these

secondary acini and considered the diverticulums or secondary acini as evidence of proliferation. A recent work of Moritz¹⁸ also favors this view. Figure 7 proves clearly that this is not the case. Glands presenting this morphology have a low weight. There is actually no budding but a formation of diverticulums through mechanical factors. However, the collapse of the follicular wall is not the only responsible factor. A modification of some of the constituents of the excretory segment also plays a rôle. In protracted cases where the thyroid has been stimulated for some time the columnar and rather dark cells gradually increase in size while the nucleus becomes large and vesicular (Type 3). The turgescence of a row of adjoining cells forces them to bulge out. The effect of the two combined factors is illustrated most clearly in Figure 6, where we notice not only depletion of the main follicle but also a bulging out of the newly formed diverticulum.

It is evident that, when this process of diverticulum formation extends along the entire follicular wall, as can be seen in Figures 4 and 8 (severe collapse), the process leads to a fragmentation of the main follicle. The key to the interpretation of senile involution of the thyroid lies in this important observation.

THE MORPHOLOGY OF INTRAFOLLICULAR COLLOID SECRETION (STORING PROCESS)

The colloid represents a storage product which in adults is the result of a very slow secreting process operating from birth. On the other hand, there is ample evidence that after acute colloid depletion the colloid can be restored quickly to normal.

One of us¹³ has carefully followed this slow accumulation of colloid in young children who died accidentally. It is a striking fact that up to the age of 12 years the high cylindrical segments are very scanty, so that the morphology of the thyroid epithelium (at this age) is almost uniform and of the low cuboidal type. As this corresponds to a gradual increase in size of the follicles brought about by a process of coalescence we concluded that low cuboidal epithelium slowly secretes colloid into the follicular cavity.

Figure 9 illustrates the appearance of the epithelium during an active process of intrafollicular secretion. The follicle represented in the center of Figure 9 has reached an extreme degree of depletion.

It belongs to a thyroid from a case of intestinal obstruction where the patient survived 4 days. A very narrow segment of high cylindrical cells is still evident. In our opinion these cells represent remaining segments of the excretory (or absorptive) function. The other cells have undergone marked changes. These cells have broadened out, their nuclei have become vesicular and increased in size and they have the characteristics of cell Type 3. They are secreting into the follicular cavity large droplets (Anderson vacuoles) and at the same time a watery, transparent fluid. We see here a striking example of the beginning of a new and very active colloid secretion. A more advanced stage is seen in Figure 10. It is from the thyroid of a child who had passed the stormy period of diphtheria and was actually convalescent. The child died suddenly of heart failure 18 days after the onset of the disease. Small doses of iodine were administered daily during the illness. The thyroid shows marked changes. The follicles are of medium size (80 to 100 microns in diameter) and much smaller than in children of the same age who die accidentally (100 to 150 microns). Most probably this thyroid underwent, during the acute stage of the disease, a period of depletion comparable to that represented in Figures 7 and 8. However, at the time of death the gland was rapidly restoring its colloid material, since almost all follicles are spherical and filled with a thin, transparent fluid. Moreover, evidence of follicle coalescence, such as Moritz¹⁸ recently described in man and Uhlenhuth⁸ in the salamander, is frequent. The newly accumulated colloid stands out in contrast to the older, denser colloid, lying in the center of the follicular acinus. Here again, as in the previous preparation (Fig. 9) the epithelium is composed of large cells with large vesicular nuclei (Type 3). If it were possible to present an unlimited number of photographs, we could easily demonstrate the frequency of similar aspects in subacute septic processes, such as peritonitis following appendicitis (7 to 9 days duration).

These observations lead us to the conclusion that the high cuboidal cells (under certain conditions large cylindrical), which contain a large hyperchromatic nucleus and clear cytoplasm with an extensive surface contact with the blood vessels, secrete the colloid into the follicle actively and rapidly. Experimental data support this view. Injection of pilocarpin into guinea pigs and rats increases the intrafollicular mass of colloid and transforms a low cuboidal

epithelium into a high cuboidal type. We also find the latter form of epithelium in rats which, having been exposed to cold, restore their colloid at room temperature. It is well known from the work of Cramer¹⁹ that exposure to cold causes a severe colloid release and it may be interesting to note incidentally that during the depletion period high cylindrical epithelium occurs, a fact that agrees with our conclusions.

The functional significance of endothelioid epithelium is obvious, namely that of a slow secretion of colloid.

THE MORPHOLOGY OF THE NORMAL THYROID

Keeping in mind the functional significance of these different types of epithelium, a survey of human thyroids of adults who died soon after accidental injuries leads to a new conception of thyroid histophysiology. The epithelium of normal control glands is in fact heterogeneous. Although the predominant type is low cuboidal, narrow segments of high columnar cells are present in some of the large follicles. If our previous observations are correct, the number and size of these columnar cell segments should give us an indication of the hormonal output of the gland. They represent the only part of the parenchyma that sets free the hormone into the circulation.

This active part is very small if we consider the fact that only one of five large follicles shows these narrow excretory segments. This observation is in harmony with the histophysiology of other endocrine glands, such as the suprarenal cortex, where the available evidence points to the fact that likewise only a very small proportion of cells is actively at work under normal conditions, while the remainder of the parenchyma is held in readiness for special emergencies.²⁰

We have come to the conclusion that in normal glands the high columnar segments can be divided into two groups. An example of the first group may be seen in Figure 1, where there are no accessory follicles. In most instances, however, we notice that they are in close contact with small secondary satellite follicles generally provided with low cuboidal epithelium and formed by a process described above (second group). These peculiarities are convincingly demonstrated in Figure 11, which shows a thyroid from a man 21 years old who was killed in a motor accident.

THE "SANDERSON POLSTERS"

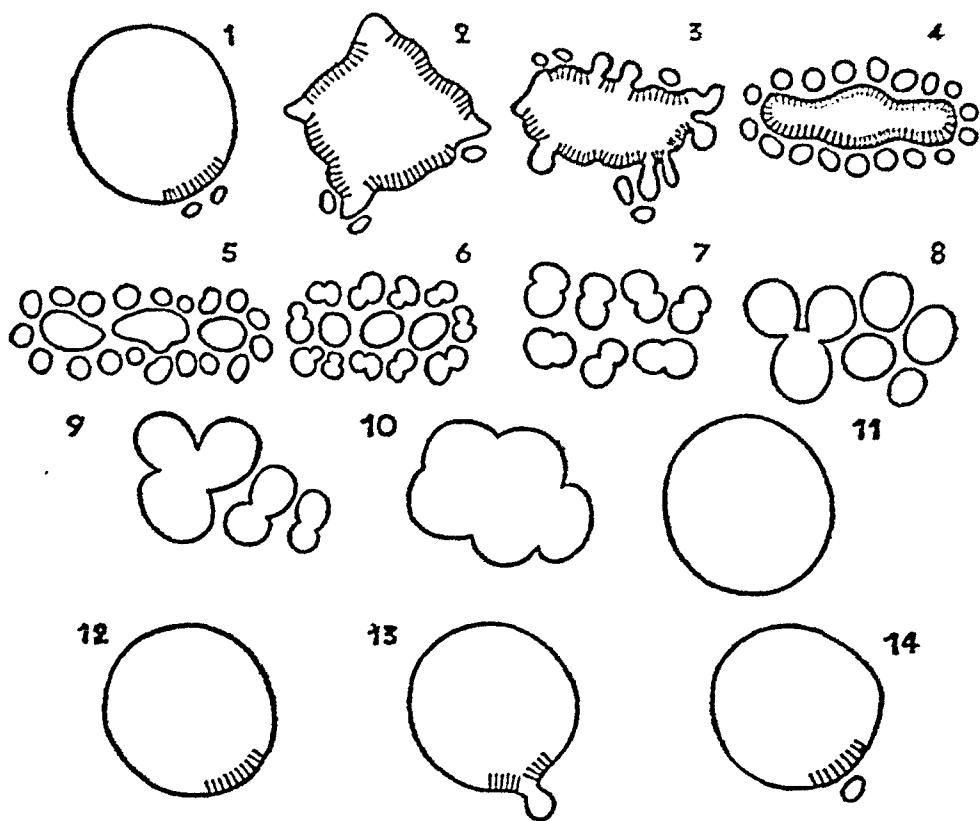
When colloid secretion increases in the small secondary follicles their mass bulges into the follicular cavity and gives rise to a "Sanderson Polster" (Fig. 2) for which we suggest the term "papilla." These secondary acini are nearly always lined with large epithelial cells (Type 3) and the nuclei are conspicuous by their size. These papillae, or "Sanderson Polsters," are a common finding in septic or toxic processes which stimulate the secretion in those secondary acini. Up to the present time the "Sanderson Polster" has been considered a center of proliferation. In our opinion it is the expression of a simultaneous stimulation of hormonal excretion by the columnar epithelium of colloid secretion by the cells with large nuclei, which are restricted to a small portion of the follicle complex. We believe that the papillary formation is due to three factors: increase in size of the cells, increase of colloid secretion and congestion of the capillaries.

These papillae consequently correspond to localized zones of functional activity, normally present, but brought into prominence by the stimulation of physiopathological conditions. In the light of these observations the significance of the small acini is quite different from that of the large follicles. Only the latter excrete the hormone into the circulation. As shown above, the two types are, however, closely related. High columnar cells having performed their excretory function become turgescient and are extruded in the form of acini out of the main follicle, the process being accentuated by the depletion of the latter.

THE FUNCTIONAL UNIT

We believe, therefore, that the human thyroid is composed of functional units, *i.e.*, complex formations of main and secondary follicles. In many cases of severe sepsis these histological features are evident and no better illustration can be given than by Figure 8. Under normal conditions the functional units are less conspicuous because the colloid storage overshadows the signs of colloid absorption. The greater part of the epithelium continues to increase the storage of colloid slowly, while in some main follicles only a small segment of cells excretes the hormone into the capillaries (Figs. 1 and 11). The concept of the functional units was brought

forward by Williamson and Pearse,¹¹ but their argument is weakened by the excessive stress they lay upon the significance of the lymphatic system, which in fact does not differ essentially from that of any other endocrine gland. The same concept has been implied by those workers who have made reconstructions of the thyroid follicle since the publication of Wilson.¹⁶ However, the only way to obtain convincing evidence is to make a study of glands profoundly changed by the physiological effect of pathological conditions.



TEXT-FIGURE 1

In health and disease the active functional units are ever-changing structures. With a constant number of cells the functional demands of the gland are met by various appropriate cell combinations.

In the early stages of a septic or toxic process the thyroid unit reacts by a stimulation of both excretion and secretion, which compensate for each other. The segments of high cylindrical cells extend, while the low cuboidal cells of the main follicle change into cells with large nuclei (Type 3), a process which brings the "Sanderson Polster" into prominence. This period of *compensated activity*

lasts a variable time. In 5 cases of streptococcic puerperal infection we found evidence of it from 8 to 10 days after the onset of fever. In peritonitis following appendicitis, in several cases of diphtheria and in protracted cases of staphylococcic pyemia, there is evidence of an early depletion. The *decompensation* is still more pronounced in intestinal obstruction. On the other hand, several cases of peritonitis following appendicitis which we have studied prove that the depletion and fragmentation can be followed by a restoration period leading, through a process of coalescence of the follicles, to the normal configuration of the functional unit (Text-Fig. 1).

The succession of periods of colloid release and colloid secretion appears as a fact of general significance in the histophysiology of the thyroid. Uhlenhuth's⁸ remarkable work proves that metamorphosis in *Ambystoma opacum* requires an activity of the thyroid which leads to a considerable colloid depletion. However, a period of active restoration of the colloid soon follows. Kuhn,²¹ on the other hand, noticed this same succession of hormonal excretion and intra-follicular colloid secretion in salamander larvae injected with thyrotropic hormone of the anterior lobe of the pituitary gland. In man (Van Goor,²² Schmelling²³) and in mammals (Benazzi²⁴) colloid release is observed in the later part of fetal life. As Benazzi has shown, the follicles at birth are already refilled when the animal is born active (such as the guinea pig or the lamb). In other mammals, such as the mouse and rat (Benazzi) and man (own observations) the colloid restoration takes place soon after birth.

DISCUSSION

We are well aware that our conclusions may lead to controversy. Any one familiar with the histology of toxic goiter will be prepared to accept the excretory function of the high columnar epithelium (*i.e.*, colloid absorption or release with hormonal output into the circulation). In fact, Holst²⁵ has already suggested that the absorption process takes place at the site of the "Sanderson Polsters." But this idea was more or less hypothetical. The critical mind will point to toxic goiters devoid of this type of epithelium. If Graves' disease or thyrotoxicosis centers entirely in the thyroid, which is far from being an established fact, our morphological studies suggest that, besides the unquestionable multiplication of its cell constituents, the thyroid shows the decompensated type of hyperfunction when no pre-

operative iodine treatment has been used. The functional units resemble in many respects those of a case of diphtheria, apart from the hyperplastic characteristics (Fig. 8). We have shown that in this type of hyperfunction a phase of colloid restoration very often follows the phase of depletion. In appendicitis there is even evidence that both processes alternate. The discrepancy in the histological findings in goiter material may be easily explained by the fact that the gland is removed during such a period of restoration. This temporary lack of thyroxin excretion need not necessarily correspond to an improvement of the symptoms, since the thyroxin is very slowly destroyed in the tissues.

There are other objections to be met. One could argue that the colloid depletion is not the result of the functional activity of columnar cells but that the morphology of the thyroid epithelium is controlled by the collapse of the follicle. This argument does not hold. In rats exposed to cold the small size of the follicles, or more exactly the difference between their diameter and the height of the cells, is so slight that collapse is not possible and yet high cylindrical cells appear at a given moment during the experiment.

In the literature columnar epithelium has generally been interpreted as a sign of hypertrophy and has been associated with cell proliferation. Marine¹⁴ says that "columnar epithelium always indicates hypertrophy." With this we cannot agree. Columnar epithelium is a normal constituent of the gland. Its scarcity explains why it has been overlooked up to the present. In any human control gland we have had no difficulty in finding columnar epithelium grouped in narrow rows. When in simple colloid goiter these columnar cells become very evident, Wegelin,⁷ Aschoff¹² and Hellwig⁵ consider it a sign of proliferation. There is no convincing argument in favor of this assertion. In our opinion the increase in number and in size of these columnar segments coincides with the onset of toxic symptoms, and indicates that the hormonal output is increased. This is much more in agreement with clinical observations. It will be interesting to reexamine simple colloid goiters from the point of view of the existence of columnar segments. If we find them regularly a much debated problem will be solved, we shall understand at last why in spite of their low cells most of these goiters are not accompanied by thyroid insufficiency. Aschoff,¹² who has based his classification partly on the existence of

the so-called proliferating buds provided with columnar epithelium, admits that the presence of the latter coincides with the appearance of symptoms of hyperthyroidism. If we compare these observations with ours, it must be conceded that they lend support to our views.

The chief argument of those who believe that high cylindrical epithelium indicates cell proliferation consists in the fact that adjacent to this type of epithelium there is always an active process of budding. That is why the Sanderson formations have been considered proliferation zones. To this we answer first of all, that in no freshly fixed human gland does one find isolated cells budding from high epithelial cells; they are always grouped in acini with a definite lumen. Secondly, we point again to the mode of formation of these secondary acini where only mechanical factors play a part.

We do not deny the possibility of a multiplication of cells in the papillae, but so far no one has given decisive proof of it. In our fresh human material we have never found mitotic figures. Of course we do not lay stress on this because we are dealing with agonal conditions. However, in thyroids of rats, guinea pigs and rabbits we have repeatedly found mitotic figures in both low and high epithelium with the same frequency. In exophthalmic goiter, where the different types of cells described above are present, mitoses are found in any one of them. What is still more convincing is the fact that the weight of the glands with a preponderance of columnar epithelium (Figs. 7 and 8) is less than the average (5 gm. instead of 7 gm. average weight; 3.5 gm. instead of 5 gm. average weight). We had the opportunity of examining several specimens of thyroids from males and females between 50 and 65 years of age killed accidentally. Extensive segments of columnar epithelium were present, as well as secondary acini. If these formations indicate proliferation why should the gland decrease in weight, as our measurements have shown? In the thyroid of salamanders Uhlenhuth noticed also that mitoses were scanty at a period when papillae with high cells were numerous.⁸

Moreover, recent observations support the view of the excretory function of columnar epithelium. As mentioned before, Van Goor²² and Schmelling²³ have proved that in the later part of human fetal life the colloid is released to a certain extent. In fresh material we find that during that period, and especially at birth, the epithelium is chiefly columnar. Moreover, in the experiments of Okkels, Krogh

and Lindberg⁹ where the hormonal excretion was measured by the basal metabolism, typical columnar epithelium appeared, as is shown in Figure 3 of their publication.

At the onset of metamorphosis of *Ambystoma opacum* the functional activity of the thyroid is unquestionable. Although Uhlenhuth⁸ does not distinguish different cell types it is evident from a perusal of his paper that columnar epithelium is present during the colloid release. We should like to call attention especially to Text-figure 16, page 645 of his paper published in *Arch. f. Entwicklungs-mechn. d. Organ*, 1927, 109. The appearance of columnar epithelium during colloid release is also strikingly demonstrated in a series of contributions by Corti's pupils on the thyroids of birds coincident with the development of feathers.^{24, 26}

We shall not discuss at length the secretory function of the cells of Type 3 the surface contact of which with the capillaries is always considerable. In fact it is with those cells as well as with the low cuboidal ones that our predecessors have been chiefly concerned. We refer to the classical work of Bensley and the remarkable contributions of Uhlenhuth.⁸ We hesitate to dwell on their cytological features for fear of interrupting the unity of our argument. More about this question will be found in a paper by one of us.¹³ We believe that Langendorff cells are compressed cells without any functional significance. Furthermore, we shall not take up the question of the inversion of polarity set forth by Cowdry or the significance of the modifications of the colloid and the Anderson intra-acinar vacuoles (Uhlenhuth,⁸ Aron²⁷) which are no doubt related to an increased activity of the gland. The Anderson vacuoles pass from the cell into the follicular cavity. Our photographs and Uhlenhuth's drawings entirely agree. The Anderson vacuoles constitute one of the aspects of rapid intrafollicular secretion and in Uhlenhuth's observations, as well as in our own, their number attains a maximum when the colloid restoration is well on the way.

It will be noticed that we have not mentioned the Wölffler cells or solid interstitial cell groups. Nothing in our extensive investigations supports their embryonic nature. In normal childhood there are no isolated interstitial cells or cell groups. They appear only when the functional units have been stimulated (see also Aschoff,¹² and Rienhoff¹⁷) and broken up into small follicles through protracted toxic or septic conditions, or through senile involution.

They are formed only when small or medium sized follicles are predominant.

We have come to the conclusion that the parafollicular cells (Nonidez²⁸) of small mammals are homologous to the small satellite follicles of the human thyroid. Mechanical conditions (small size of the follicle) prevent the collapse and folding of the follicle wall and only the second mechanical factor mentioned previously plays a part, namely the turgescence of the cell. The process has been clearly described by Florentin²⁹ and Nonidez.²⁸

Bernard,³⁰ Benazzi²⁴ and Florentin²⁹ have suggested that these cells or cell groups excrete the hormone actively into the blood stream. Examination of material from small mammals may favor this view, as does also the cytological structure of these cells and their close connection with the blood vessels. However, a wide experience with the human thyroid compels us to discard entirely this opinion. Furthermore, the predominant histological features of toxic goiter are completely opposed to this theory.

CONCLUSIONS

1. Four types of epithelium exist in the normal human thyroid: (1) the low cuboidal type which secretes colloid slowly into the follicular cavity; (2) the large high cuboidal or broad cylindrical cell type with large nuclei which secretes colloid actively into the follicular cavity; (3) the columnar type which absorbs the stored colloid and excretes the hormone into the blood or lymph circulation; and finally (4) the endothelioid type which is associated with a very slow colloid secretion.

2. The thyroid cells are grouped in ever changing functional units which under normal conditions differ from each other by their hormonal output.

3. The units containing narrow columnar cell segments (a normal constituent of the gland) excrete the hormone actively into the circulation.

4. These active functional units are composed of a main follicle and satellite follicles. The excreting zone of columnar cells is always located in the main follicle and usually in close connection with satellite follicles. The remaining epithelium of the functional unit is for the most part low cuboidal and under certain conditions

endothelioid. Occasionally some cells with large nuclei are present in the satellite follicles.

5. Under normal conditions the amount of stored colloid is kept fairly constant by the compensatory activity of columnar epithelium on the one hand and cuboidal epithelium on the other.

6. The small satellite follicles are derived from the excretory segment, not as the result of a budding process but through the action of two mechanical factors: (1) the folding of the excretory epithelium following depletion, and (2) the turgescence of the columnar cells that have exhausted their excretory function. The latter factor may operate alone. The small satellite or secondary follicles represent cell groups beginning anew their functional cycle.

7. There are no interstitial cells or solid interstitial cell groups present in the normal infant or adult thyroid, while in senile involution they may become apparent. This observation refutes the embryonic nature of these elements.

8. "Sanderson Polsters" are the result of functional stimulation of the excretory zone which expands, and of the group of underlying acini which secrete colloid more actively. This papillary formation is caused by an increase in size of the cells, an increase of the colloid secretion and vasodilatation.

9. Although we do not deny the possibility of cell multiplication in high columnar segments or in the "Sanderson Polsters" we maintain that they are not specifically proliferation centers under normal conditions.

10. A very small proportion of thyroid cells excretes hormone into the circulation.

11. Under pathological conditions the gland reacts at the beginning by an extension of the columnar excretory segments and by the transformation of the low cuboidal type into the cell type with large nuclei. At this stage there is no depletion. Increased hormonal excretion into the circulation is compensated for by increased intrafollicular secretion. This compensated stage may last 10 days (streptococcic septicemia). In cases of peritonitis following appendicitis, intestinal obstruction, and occasionally in diphtheria, there is evidence of an early decompensation, the excretion predominating over the intrafollicular secretion.

12. The depletion of the main follicle leads to the formation of secondary acini and finally to extensive fragmentation of the func-

tional unit. The latter may be restored to its normal features through the secretory activity of the small follicles which fuse together. There is evidence of alternating periods of colloid release and colloid storage during protracted infectious or septic diseases.

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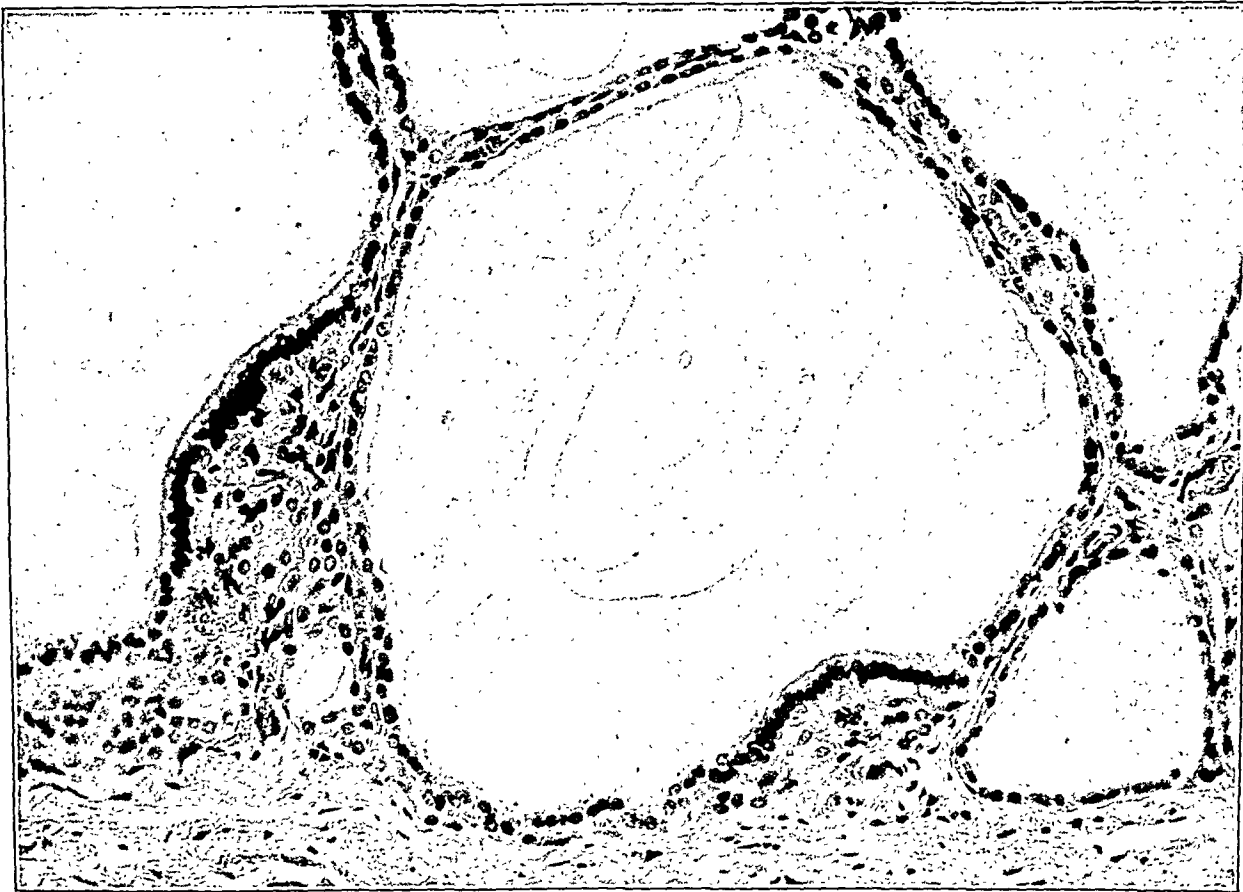
DESCRIPTION OF PLATES

We are indebted to Mr. F. Pittock for the photomicrographs. These were made through the kindness of Professor J. P. Hill, of the Department of Histology, University College, London.

PLATE 153

FIG. 1. Thyroid from a woman 54 years old. Intestinal obstruction by strangulated umbilical hernia. Survival 2 days. Distended follicle. Heterogeneous epithelial lining. Narrow columnar segment slightly protruding into the follicular cavity. Turgescence of the nuclei of the underlying sinusoids. $\times 300$.

FIG. 2. Follicle from the same case showing slight colloid release. Four types of epithelium — endothelioid, low cuboidal, cells with large nuclei and columnar. The columnar is larger than in Fig. 1. Vasodilatation of the underlying capillaries, satellite follicles. "Sanderson Polster" protruding into the follicular cavity. $\times 300$.



I



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PLATE 154

FIG. 3. Thyroid from the same case at lower magnification showing the correlation between the extension of columnar epithelium and colloid depletion. $\times 150$.

FIG. 4. Thyroid from a woman 70 years old. Intestinal obstruction caused by a scirrhus carcinoma of the sigmoid colon. Survival 10 days. Advanced stage of colloid depletion. The follicle is surrounded by uniform columnar epithelium. Note apical colloid granules. Marked formation of diverticula and satellite follicles provided with a lining of cells with large nuclei (Type 3). $\times 500$.



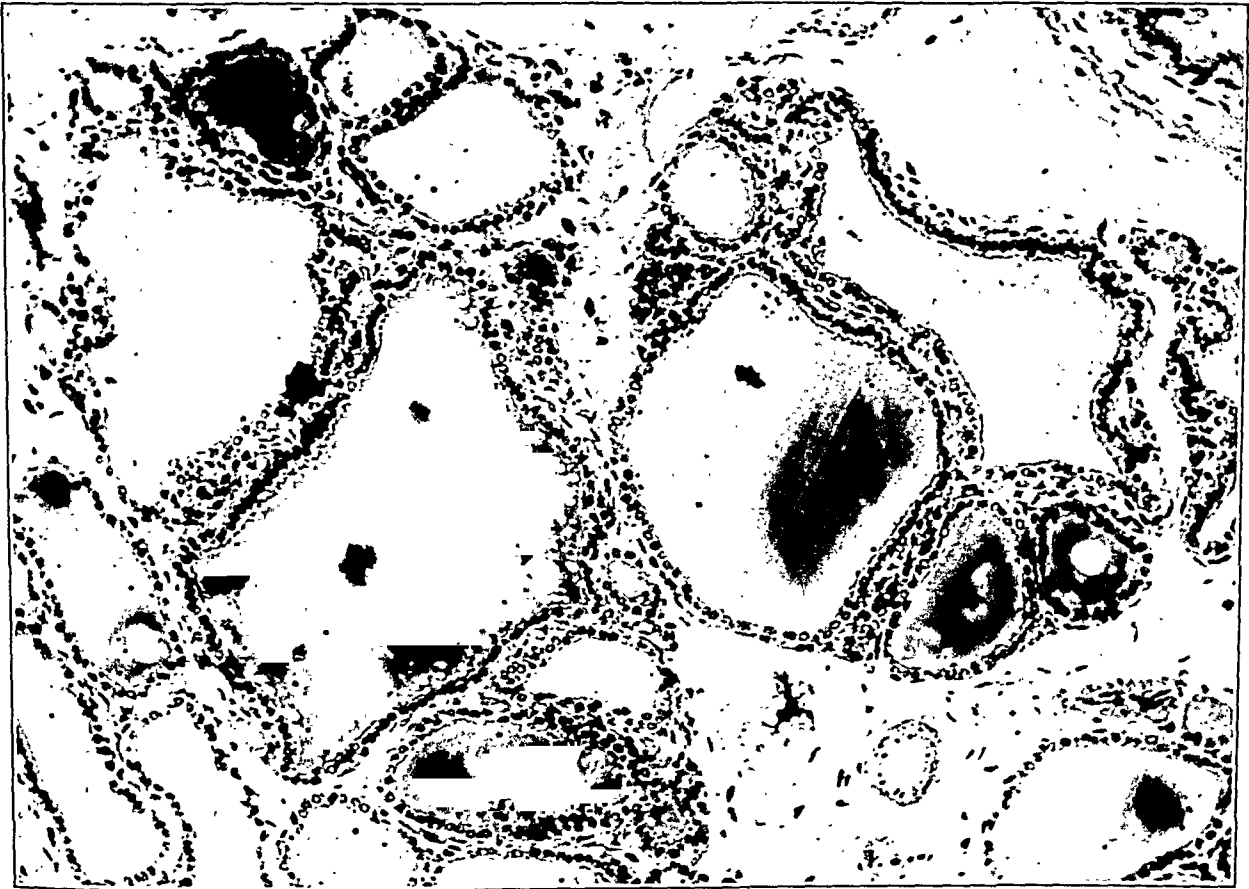
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PLATE 155

- FIG. 5. Thyroid from a girl 7 years old. Diphtheria, toxic symptoms, glomerulonephritis. Region of the thyroid showing the correlation between the extension of columnar epithelium and colloid depletion. $\times 230$.
- FIG. 6. Thyroid from a woman 54 years old. Intestinal obstruction. Morphological features of diverticulum formation (cells with large nuclei). $\times 230$.
- FIG. 7. Thyroid from a girl 7 years old. Diphtheria. Acute clinical course. Severe depletion of a follicle. Diverticulum formation. Note the clear zone at the bottom of each cell. Edema of the stroma. $\times 300$.



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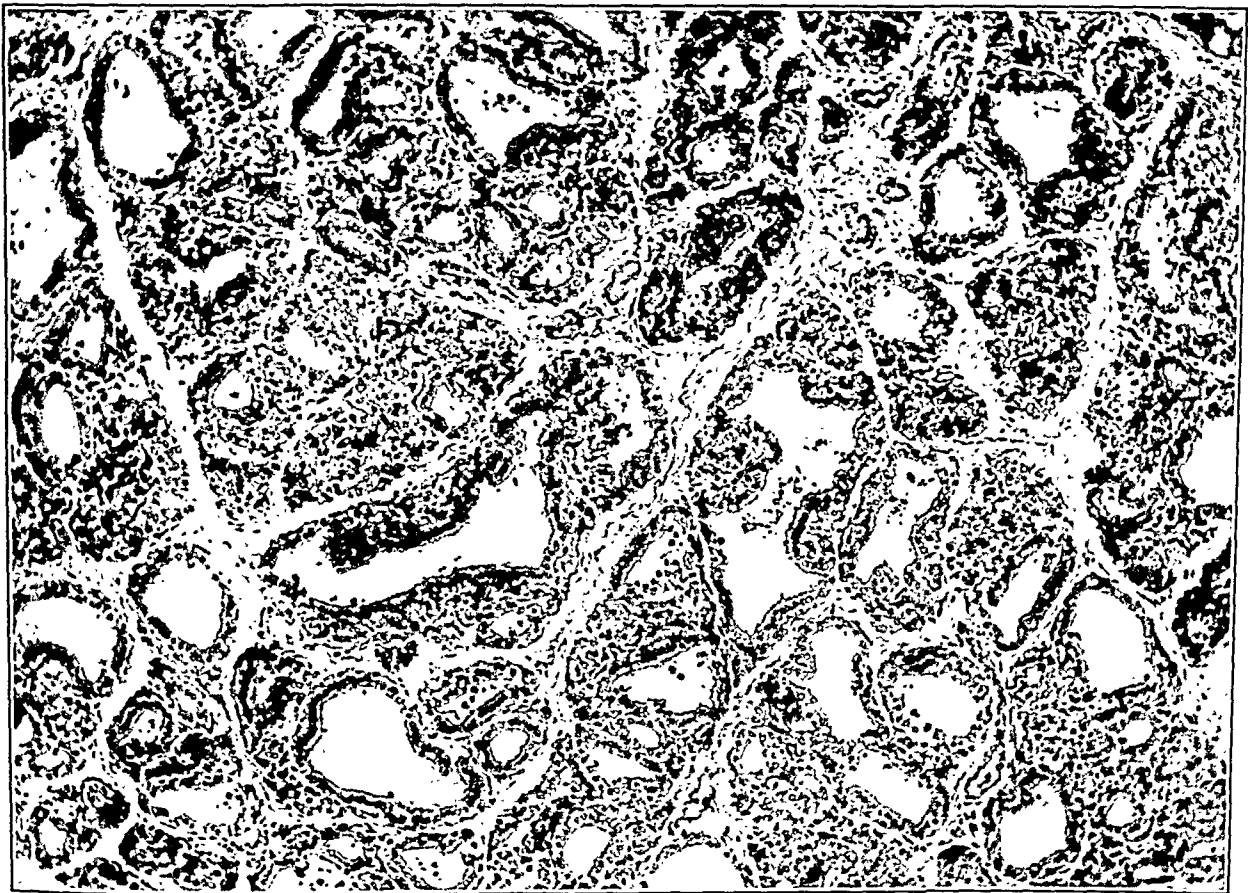


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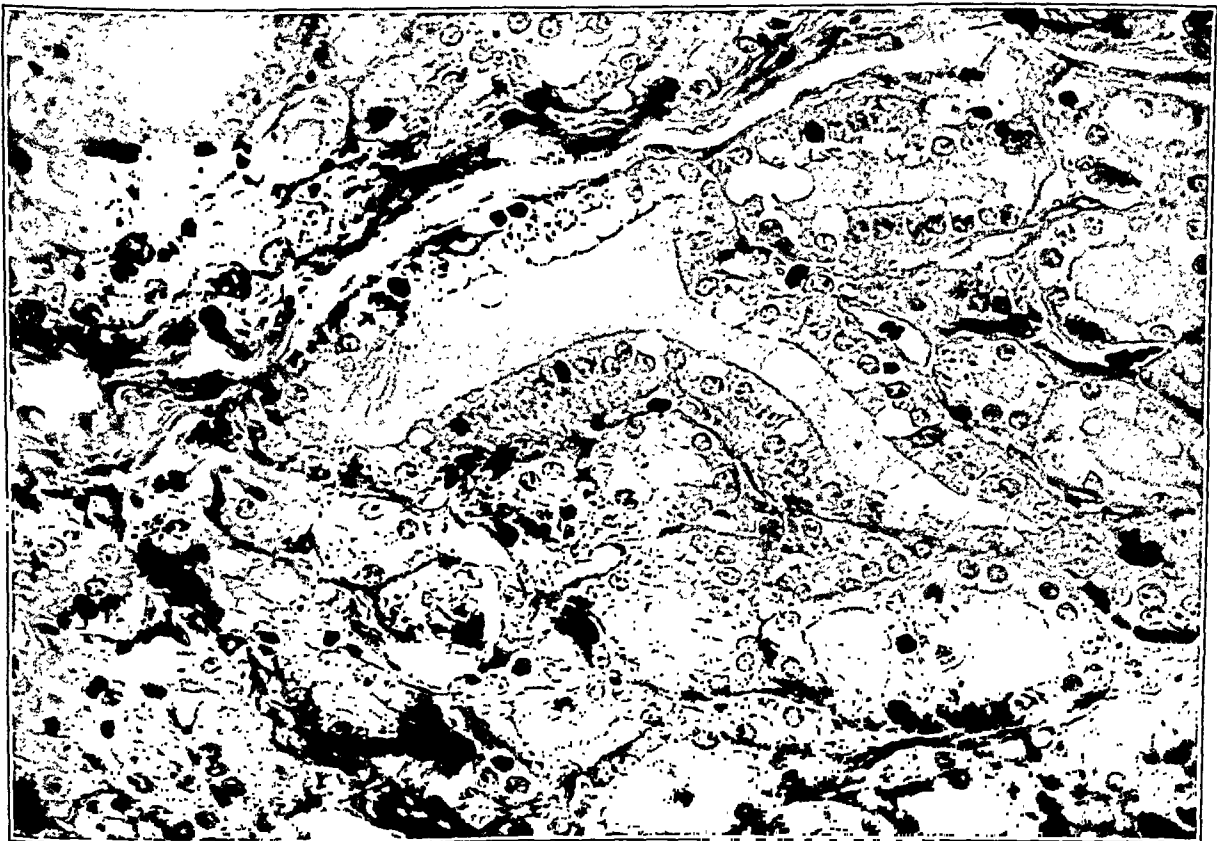
PLATE 156

FIG. 8. Thyroid from a girl 5 years old. Acute diphtheria. Severe colloid depletion. Extensive development of columnar epithelium. Marked formation of satellite follicles. Functional units very evident. $\times 150$.

FIG. 9. Thyroid from a woman 57 years old. Intestinal obstruction by strangulated umbilical hernia. Survival 4 days. In the center of the photomicrograph is a collapsed follicle filled with a clear colloid. Note a narrow columnar segment. The remainder of the epithelial lining is composed of cells with large nuclei. Secretion of apical vacuoles (neosecretion). $\times 500$.



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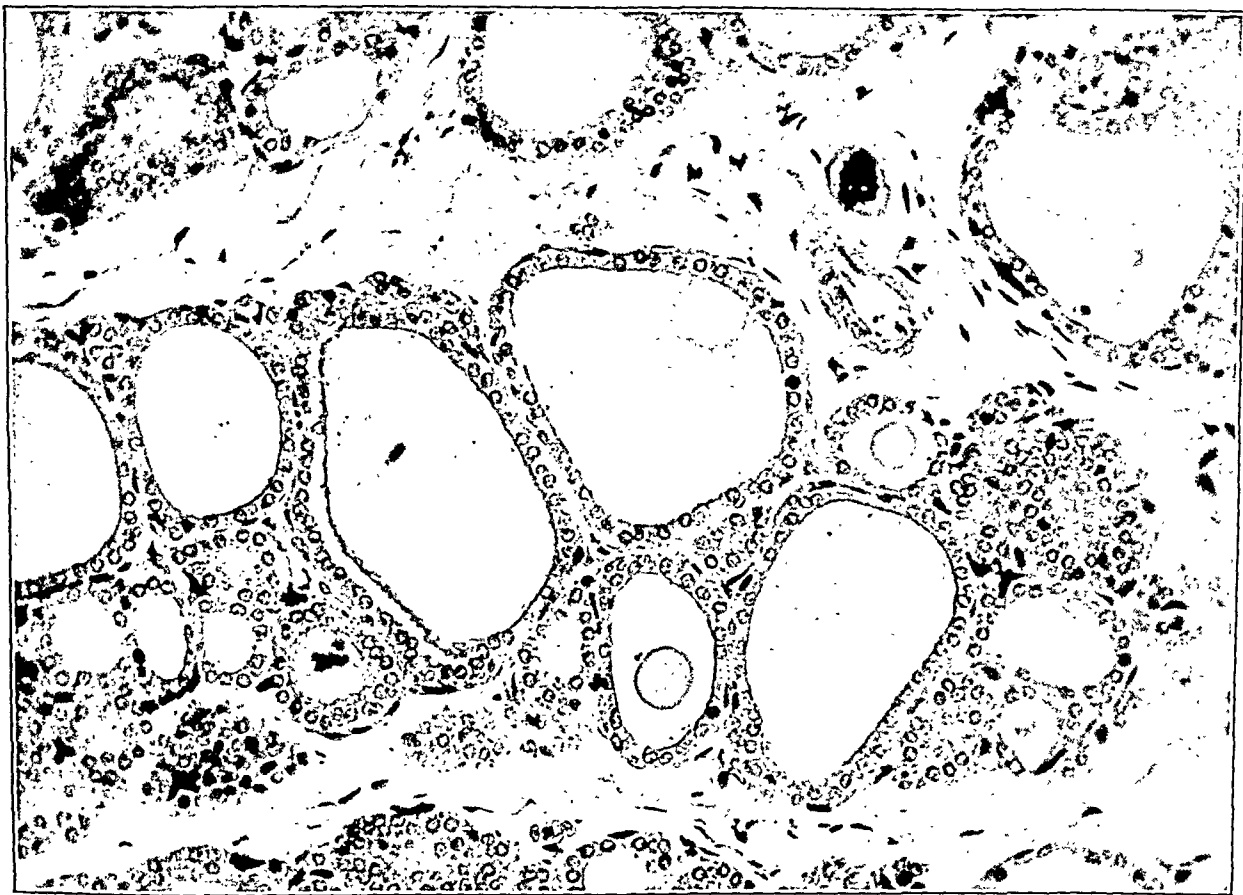


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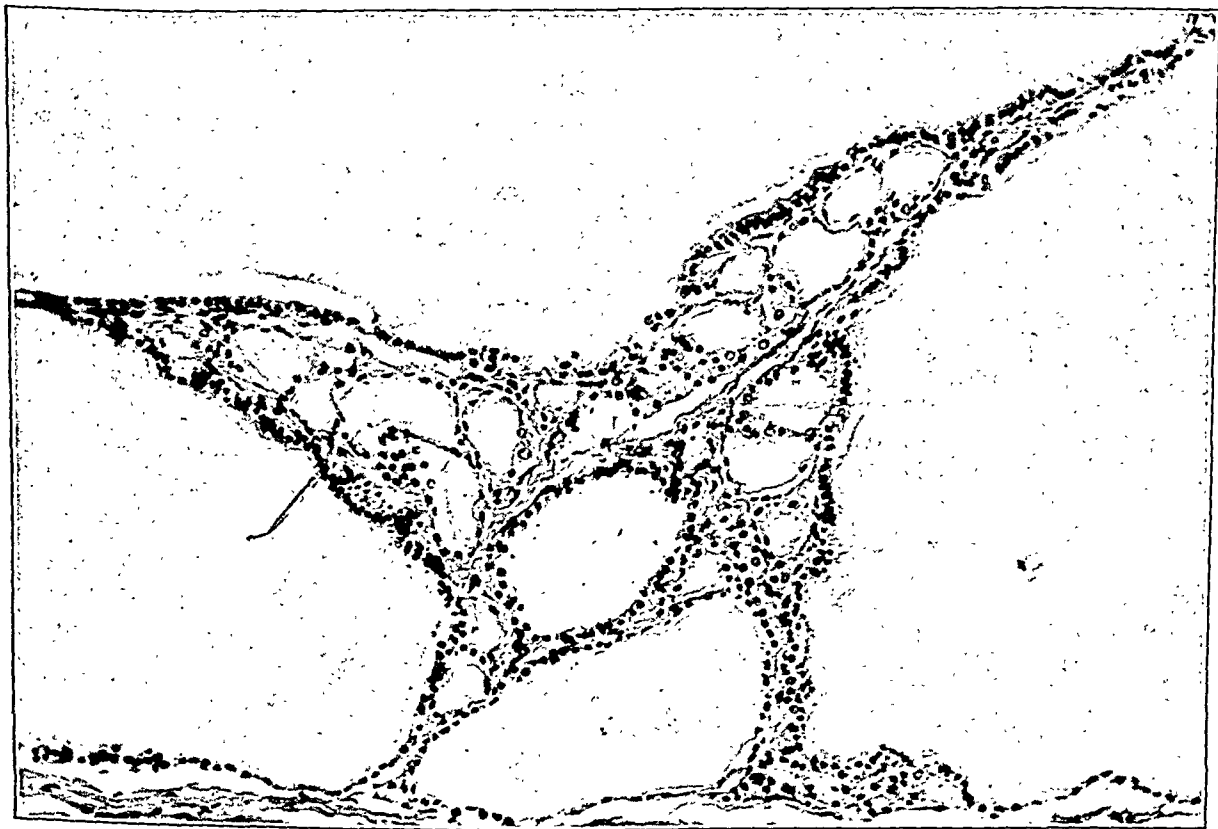
PLATE 157

FIG. 10. Thyroid from a girl 9 years old who died suddenly during recovery from diphtheria. Stage of restoration of the colloid. The old, denser colloid can be seen in several follicles. Uniform aspect of the epithelial lining of the distended follicles (cells with large nuclei — Type 3). $\times 300$.

FIG. 11. Thyroid from man 21 years old. Fracture of the skull. Survival 48 hours. Specimen not fresh. Note the three excretory segments (columnar epithelium) belonging to three adjoining functional units, and the group of satellite follicles which lies under each columnar segment. $\times 230$.



10



11

A UNIQUE INFECTION IN MAN CAUSED BY A NEW YEAST-LIKE
ORGANISM, A PATHOGENIC MEMBER OF THE
GENUS SEPEDONIUM *

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A middle-aged white man who had had an irretractable skin disease for 15 years attracted our attention because the extent and the gross features of the lesions were unlike anything we had ever observed. A survey of the literature convinced us that the clinical manifestations of the ailment indicated a new disease. Unique papular lesions with a small crater of necrosis capping each, which yielded a few drops of sticky pus, covered the entire skin. The lesions were heavily set on a much thickened, wrinkled and scaly skin, which had a tendency to become ulcerated. The ulcers were deep and crusted and progressed slowly. The regional lymph nodes were increased markedly in size. The features enumerated led us to believe that a moderately strong injurious agent was the cause of the disease. The extension of the lesions over the entire surface of the body, followed by enlargement of the regional lymph nodes, led us to suspect an infectious form of microbiological life, possibly a fungus, as the etiological agent of the disease. Sections of the pathological skin and lymph nodes revealed the presence of minute yeast-like bodies chiefly within endothelial phagocytes. The organism was isolated on artificial culture mediums. Mycological and animal pathogenicity studies of the pure culture were made. According to the taxonomy of Saccardo, the organism belonged to the genus *Sepedonium*. Our species did not belong to any of the described members of the genus. No pathogenic member of the *Sepedonium* has been described.

REPORT OF CASE

Clinical History: H. J., a steel welder, was observed on Feb. 9, 1931. He complained of a generalized skin eruption which was accompanied by intense itching. In 1917 dry scaly skin lesions developed in the regions of the popliteal

* Received for publication June 21, 1934.

spaces from which the remainder of the skin eventually became involved. He had been a sailor for 3 years, a railroad switchman for several years, and a welder of steel flues for the past 14 years. No flux or brass was used and the shop in which he worked was well ventilated. In September, 1929, lesions began to appear on the thighs. Progression of the lesions continued until he was unable to work because of involvement of the palms of the hands.

Physical examination revealed a man whose general condition was quite good. He had a generalized, dry, papular skin eruption which caused him considerable distress from itching. One buccal lesion was observed. Many types of local and general therapeutic agents were tried but these served only to relieve symptoms and had no effect upon the progress of the disease. He was discharged on March 29, 1931, with a diagnosis of dermatitis exfoliativa.

The patient was rehospitalized on July 7, 1932. Since his last hospitalization, ending on March 29, 1931, he had visited numerous physicians and health resorts but had received no aid. He had received a course of eight X-ray treatments in September, 1931, with no benefit, and a biopsy of the skin and an inguinal lymph gland was made in the institution where he had received the X-ray treatments. A diagnosis of dermatitis and lymphadenitis was made, but because of the heavy infiltration of the skin with lymphoid cells the question of leukemia of the skin was raised. Until May, 1932, the skin of his back was fairly smooth but since then the old lesions became more elevated and many new papules appeared. This was accompanied by loss of weight and weakness. He had difficulty in keeping warm even during the summer months. The right small toe became gangrenous and sloughed off in July, 1931. Several fingers of the right hand had been lost in an accident.

Physical examination revealed a weak, emaciated male, whose entire skin was involved in an extensive dermatitis. The lesions varied from scaliness and papule formation to ulcerated lesions measuring 3 to 4 cm. in diameter. The most recent lesions were papules measuring 0.5 to 1 cm. in diameter, which were somewhat irregular in outline and had a tendency to become confluent. The pruritus was intense. The thickened mucous membrane of the mouth presented several small, granular, ulcerated lesions. The heart, lungs and abdominal viscera were normal.

Laboratory Studies: The urine was normal. Red blood cells 4,200,000; white blood cells 10,200; hemoglobin 82. Blood Wassermann negative. Blood studies for evidence of changes in the bleeding time, coagulation time, fragility of the red blood cells, clot retractibility and prothrombin time showed no abnormalities. The blood platelets were 0.35 per cent (Van Allen). The CO₂ combining power of the plasma was 60.7. No lesions were demonstrable roentgenologically in the chest, or dorsal or lumbar vertebrae. Biopsies of the skin, the left inguinal lymph gland and the buccal mucosa were made on July 9, 1932. Each of these tissues was cultured and the yeast-like organism isolated. The tissue sections revealed definite evidence of a chronic inflammatory process and the presence of numerous yeast-like bodies in phagocytes. A few were free in the tissue and in the epithelial cells.

Therapy consisted of massive doses of potassium iodide and ionized copper treatment. X-ray was also applied to a small area of the lesions, all of which seemed to progress under treatment.

Under observation the patient developed new skin lesions and a punched-out ulcer of the tongue. Many of the older firm papules became ulcerated. His temperature, which had been of a mild septic type since admission, gradually

became more elevated. Three days before death friction rubs and râles were heard in the chest. Death occurred Aug. 7, 1932, presumably 15 years after the onset of the disease.

POSTMORTEM EXAMINATION

The body was that of a well developed, emaciated male, whose entire skin was thickly studded with papular and macular lesions measuring 0.5 to 1 cm. in diameter. Many deep ulcers from 1 to 4 cm. in diameter were found, as well as numerous shallow ulcerations which represented the recent liquefaction of the summits of papules. Large decubitus ulcers were present over the sacral and scapular regions. The skin was thickened, reddish purple in color and fissured in many areas. No areas of the skin escaped involvement. The lesions were found in the scalp, eyebrows, palms of the hands, soles of the feet and the scrotal sac, as well as over the larger skin surfaces. Papular and ulcerated lesions were also found on the roof of the mouth, the tongue and the mucous membranes of the cheek.

Except for fibrous adhesions between the gall-bladder and the colon, the peritoneal cavity appeared normal. The left pleural cavity contained 150 cc. of cloudy sanguineous fluid. Fibrin covered the pleura. The right pleural cavity appeared normal. The lower lobes of both lungs were firm and not crepitant. The cut surfaces were granular and yielded a purulent fluid. The medium sized vessels were occluded by red, friable blood clots. The heart weighed 360 gm. No endocardial lesions were present. The spleen appeared somewhat fibrotic. The liver weighed 2670 gm. The cut surface had a yellowish color and yielded an abundance of greasy material. The gastro-intestinal tract, gall-bladder, pancreas and kidneys appeared normal. The adrenals were somewhat enlarged and showed small areas of necrosis in the medulla. Numerous atheromas and ulcerations were present on the intima of the aorta. The bladder and prostate appeared normal. The brain showed no lesions. All superficial lymph nodes were firm and enlarged, the largest being about 4 cm. in diameter.

HISTOLOGICAL EXAMINATION

Except for slight myocardial scarring, the heart appeared normal. Sections obtained from the lower lobes of the lungs showed the alveolar sacs filled with polymorphonuclear leukocytes, fibrin and red blood cells. Many bronchioles showed partial or complete destruc-

tion of their walls. Several of the smaller branches of the pulmonary artery were occluded by organizing thrombi which showed canalization at their peripheries. Both old and recent infarcts were present. Occasionally yeast-like bodies were found which were engulfed by large mononuclear phagocytes. These organisms were found in the alveolar sacs and not in well defined lesions, as observed in the skin and the adrenals. The yeast-like bodies were not numerous. A few spiculated forms of the infectious agent were present. The spleen showed some thickening of the sinusoidal walls. The malpighian corpuscles were atrophic and many plasma cells were noted throughout the pulp. No lesions due to the presence of the organism were noted. The pancreas and gall-bladder appeared normal. The liver showed a moderate fibrosis and a chronic inflammatory cell infiltration of the supporting connective tissue of the portal canals. Fatty metamorphosis of the periphery of the lobules was present and a few small areas of focal necrosis were noted. The kidneys, except for a few small arteriosclerotic infarcts, appeared normal. The adrenals presented medullary and cortical areas of caseation necrosis resembling those produced by *Mycobacterium tuberculosis*. A few yeast-like bodies were present in the central portion of these caseous areas but they were found in great abundance in the phagocytes and the adrenal cells at the periphery. Myriads of microorganisms were found in the adrenal cells proper, in areas where no necrosis had as yet occurred. This was a conspicuous finding in all the early lesions. The largest adrenal lesion measured about 0.2 cm. in diameter. Large subintimal deposits of atheromatous material were observed in the aorta. Organizing thrombi were attached to the bed of some of the atheromatous ulcers. The skin sections taken before and after death, as well as the sections taken at another institution in September, 1931, revealed large numbers of microorganisms engulfed by large mononuclear leukocytes. A papule consisted of a collection of large mononuclear phagocytes located directly beneath the epidermis and confined to the corium. The infectious agent caused very little connective tissue proliferation. The larger, non-ulcerated lesions showed only slight evidence of liquefaction necrosis. Up to twenty or twenty-five rounded organisms, each surrounded by a distinct capsule, were contained within one phagocyte. A few organisms were noted in the epithelial cells themselves. Sections of the lymph glands, which were obtained before and after death, as well as the one removed elsewhere in September 1931, also revealed large

numbers of organisms. Scarring distorted the architecture of the glands so that no evidence of germinal centers was observed. Large numbers of plasma cells were present and a hyperplasia of the endothelial cells was in evidence. The latter contained the organisms. In a few instances collections of phagocytes heavily laden with yeast-like bodies were observed but as a rule they were scattered diffusely throughout the gland. Giant cells were abundant in the lymph glands, as compared to the other tissues involved, but they were not a prominent feature in any of the tissues. They contained relatively few organisms. The brain sections revealed no pathological changes.

MYCOLOGICAL STUDY

The appearance of the organisms in the tissues was that of rounded, yeast-like bodies. They varied little in size, and together with the chitinous fungus-cellulose capsule measured from 3 to 5 microns in diameter. There were a few swollen hyaline bodies which were judged to be empty capsules. The organisms were, for the most part, enclosed in monocytes but many were present in adrenal cells proper. A few were free in the tissues and some were present in epithelial cells of the epidermis and in giant cells. They were very numerous in injured adrenal cells at the periphery of a lesion. The organism stained better with various modifications of hematoxylin, (phosphotungstic acid hematoxylin and iron hematoxylin) than they did by the Giemsa method. In the lungs the organisms were larger than they were in the lymph nodes, the skin and the adrenals, measuring about 6 microns in diameter. Some of the yeast-like bodies in the lungs had a thick spiculated capsule, such as was later seen on artificial culture medium.

A biopsy of skin and lymph node was made on July 21, 1932. The tissue from each site was divided into two equal portions. One portion was washed in 6 changes of sterile broth. The other half was first treated momentarily with alcohol and then washed in 6 changes of sterile broth. The pieces of tissue were then macerated in a small amount of broth and test tubes of medium were then inoculated. What remained of the macerated tissue after inoculation of artificial mediums was inoculated into the peritoneal cavities of guinea pigs and mice. No evidence of disease developed in the animals after several months of observation.

Meat infusion agar, blood agar, brain agar, chocolate agar, Sabouraud's medium, 25 per cent rabbit blood agar, and beer-wort

agar were employed. The inoculum of a single loop was passed down a series of several large tubes of medium. The number of contaminating organisms was thus diminished so that isolated colonies of the fungus were readily obtained. In 7 days, there being no particular food requirement for the organism, numerous, small, flat, arborescent, icy-appearing colonies were barely visible on many of the tubes. At times there was no contamination, the tube being thickly set with colonies of the fungus. Even before the colonies appeared, smears from the surface of the medium revealed mycelial threads springing from the yeast-like bodies in a phagocyte. There seemed to be little difference in the rapidity of growth between the temperature range of 22° to 38° C. Under anaerobic conditions the growth was retarded. Colonies appeared first regularly on meat infusion and blood agar. On these mediums mycelial threads were more abundant. This was particularly true of the meat infusion agar. The growth on the mediums with a high percentage of serum and the mediums cultivated under anaerobic conditions developed more slowly, was butter-like in consistence and was composed almost entirely of large, round, yeast-like bodies.

Hanging drop cultures showed branching septate mycelium with the development of a large spore within a long mycelial thread, but much more frequently these spores were found within and at the end of a short mycelial branch. The surface of the spore was at first smooth, but later it took on a distinct spiculated appearance. Conidiospores were observed and there was no dissemination, indicating that the spores were well contained within the mycelial threads. Round, hyaline bodies, which were quite uniform in size and appeared as spores, were frequently seen within these large chlamydospores. They disappeared upon heating and absorbed fuchsin and sudan III. We were convinced of their lipoidal nature. These globules were especially abundant in cultures containing serum or those grown under anaerobic conditions. None of the globules survived fixation and staining methods.

Colonies grown directly on cover glasses showed a thallus of delicate mycelium. The flexibility of the filament is interpreted by the way its direction of growth is diverted when it encounters even the smallest particle on the cover slip. When a mycelium branches, the angle formed between the two mycelial threads is usually more than 45°. Usually after 10 days the large spiculated chlamydospores develop within and at the ends of the short lateral branches near the

center of the thallus. These spores extend peripherally as the thallus grows. After 3 weeks spores are about all that is left of the thallus. The mycelia are for the most part degenerated.

The mycological study in our case included a consideration of the various organisms which appear in tissue as yeast-like bodies. Leishman-Donovan bodies, Darling's *Histoplasma capsulatum*, *Oidium gilchristii*, *Monilia albicans*, *Coccidioides immitis*, *Torula histolytica*, *Phialophora verrucosa*, and the organism of pseudofarcy were compared with our organism. It appeared somewhat like Leishman-Donovan bodies in tissue. Giemsa's stain failed to bring out a kinetic nucleus. Unlike the organism of leishmaniasis, it was not pear-shaped and the chitinous cellulose material about the nuclear substance indicated a fungus rather than an animal parasite. The size of the organism in our case suggested the *Histoplasma capsulatum* of Darling, more than any of the above mentioned yeast-like organisms. It was much smaller than the other yeast-like bodies which appear in tissue. However, in the case here reported, there was no involvement of the spleen and the organism was culturally unlike the organism of pseudofarcy which has been presumed to be similar to Darling's *Histoplasma capsulatum*. The cultural characteristics were also quite unlike any of the above named organisms which appear in tissue as yeast-like bodies.

ANIMAL PATHOGENICITY

Inoculation of macerated skin and lymph nodes into the peritoneal cavity of mice and guinea pigs produced no disease. The inoculation of guinea pigs and rabbits subcutaneously with the isolated fungus resulted in local lesions after 7 days. The lesions progressed for 7 days, when definitive evidence of regression was noted. The animals were killed after approximately 4 weeks. The organism was still alive in the lesion but there was no dissemination of the infection and it seemed definite that the lesions would have healed in these animals. The dog and the rat developed progressive lesions. The animals were killed, but judging from the extensive lesions in the lungs, spleen, adrenals and liver, it appeared relatively certain that these animals would have died of their generalized infection. The small yeast-like bodies were found in the granulomatous lesions of these experimental animals. Pure cultures of the yeast-like organism were isolated from the lesions 3 to 4 weeks after the inoculation of the animal.

SUMMARY AND CONCLUSIONS

A case of a chronic infection produced by a yeast-like organism belonging to the genus *Sepedonium* has been reported. The infectious agent was apparently localized in the skin and the regional lymph nodes for a period of about 15 years. The skin was thickened and scaly throughout the course of the disease, except during the last 3 months of life when the characteristic papular lesions developed. It is possible that this fungus infection could have been a secondary infection ingrafted upon a non-specific scaly dermatitis, but the presence of the yeast-like organism in the skin and lymph glands for at least a year and a half before the lesions became papular, and the fact that the enlargement of the lymph nodes was an early observation, make this possibility seem quite improbable. It is our opinion that the disease was initiated by the fungus.

The appearance of the organism in tissue, the large spiculated chlamydospores on artificial culture medium and the animal pathogenicity of the organism are the characteristic features by which subsequent cases may be recognized.

The infecting organism is similar in the chronicity of the infection it produced, the macroscopic appearance of its growth upon artificial culture medium and the formation of spores upon lateral branches to the so-called oidium mentioned in medical literature. However, the large spiculated spores, the delicate mycelium and the animal pathogenicity are distinctly different from the *Oidium gilchristii*.

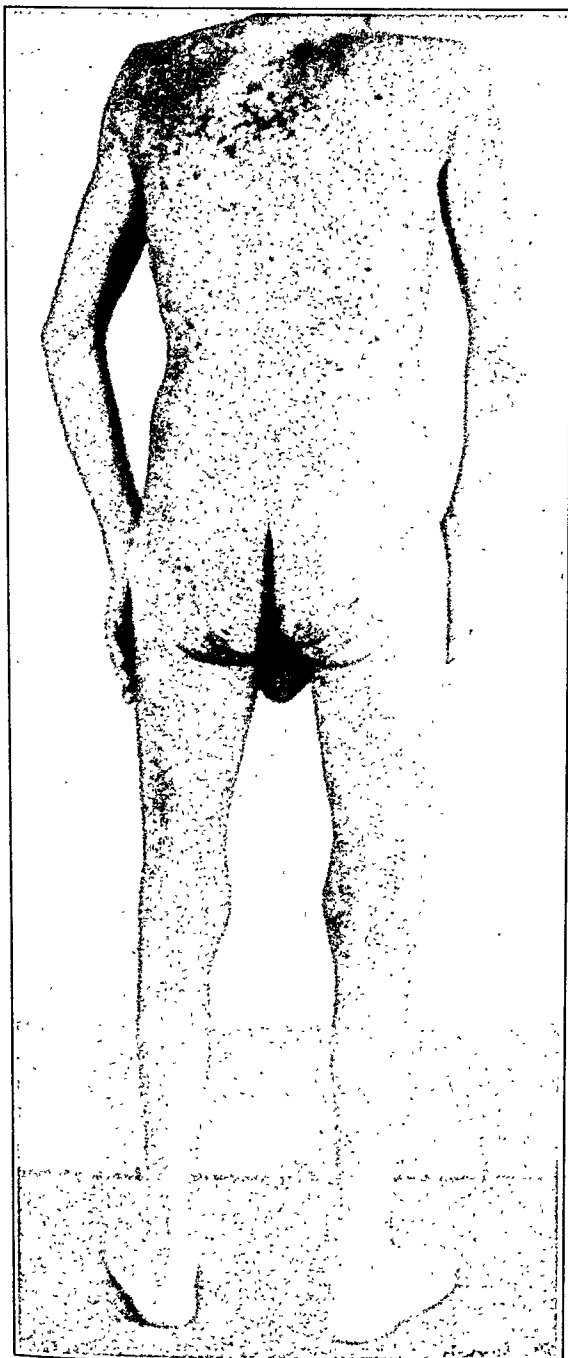
Although we appreciate that the taxonomy of this large group of imperfect fungi, to which this organism belongs, is artificial and often very unsatisfactory, it would appear that this organism could not be more satisfactorily classified for the present than with the genus *Sepedonium*, since no spore formation from the copulation of hyphae was observed.

DESCRIPTION OF PLATES

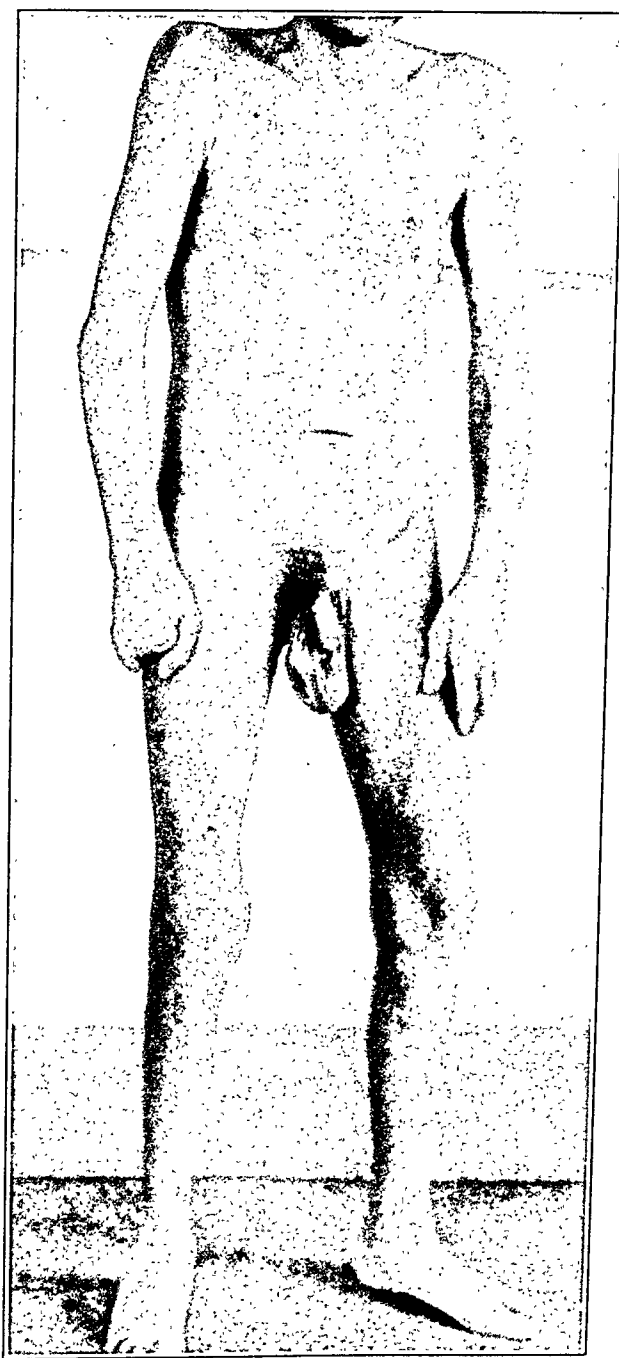
PLATE 158

FIGS. 1 and 2. Photographs showing the distribution and nature of the skin lesions.

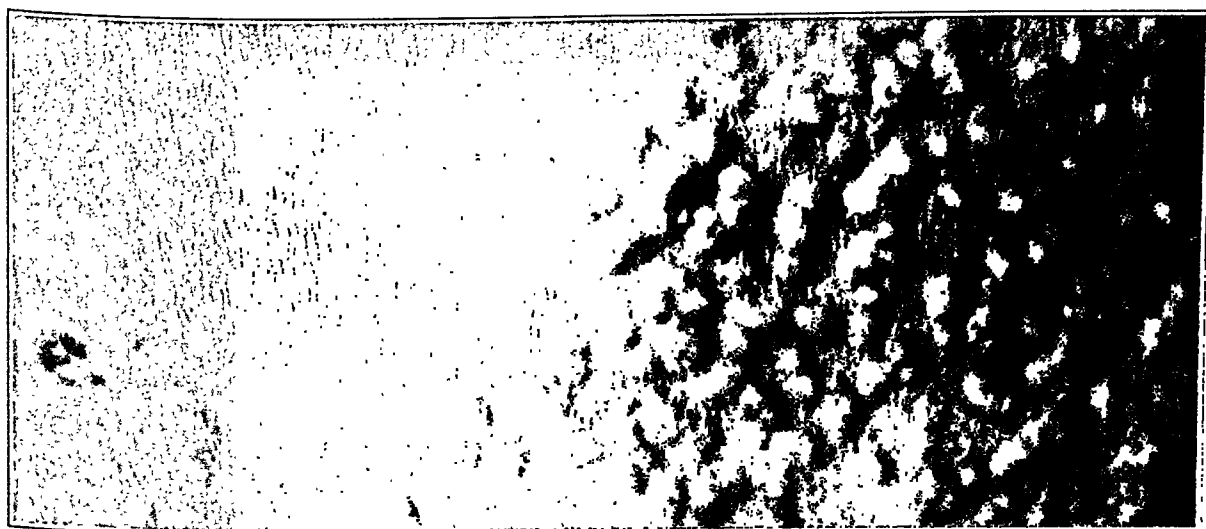
FIG. 3. Photograph of the thickened, wrinkled, scaly skin which shows many papules with crater-like ulcerations of their summits.



I



2

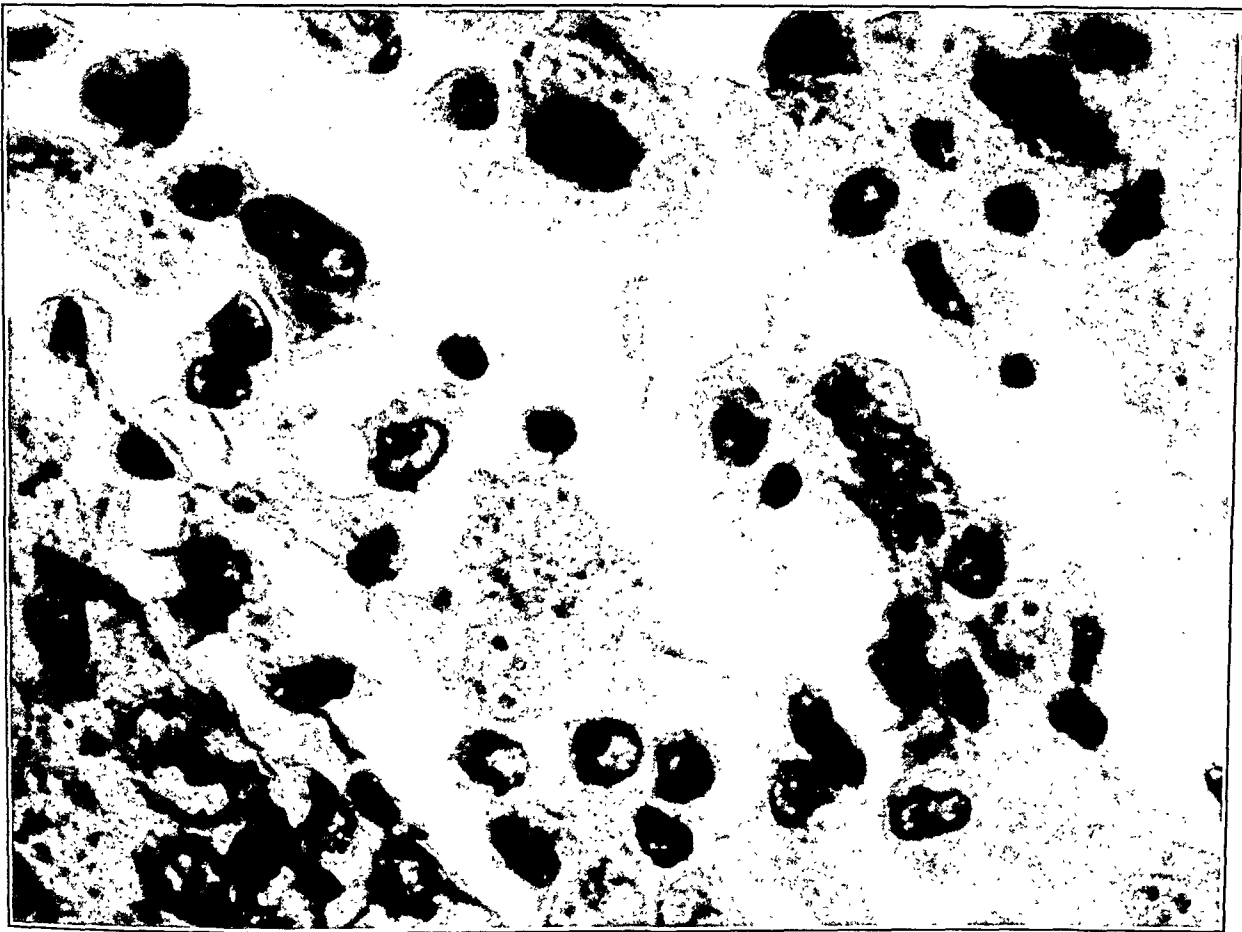


3

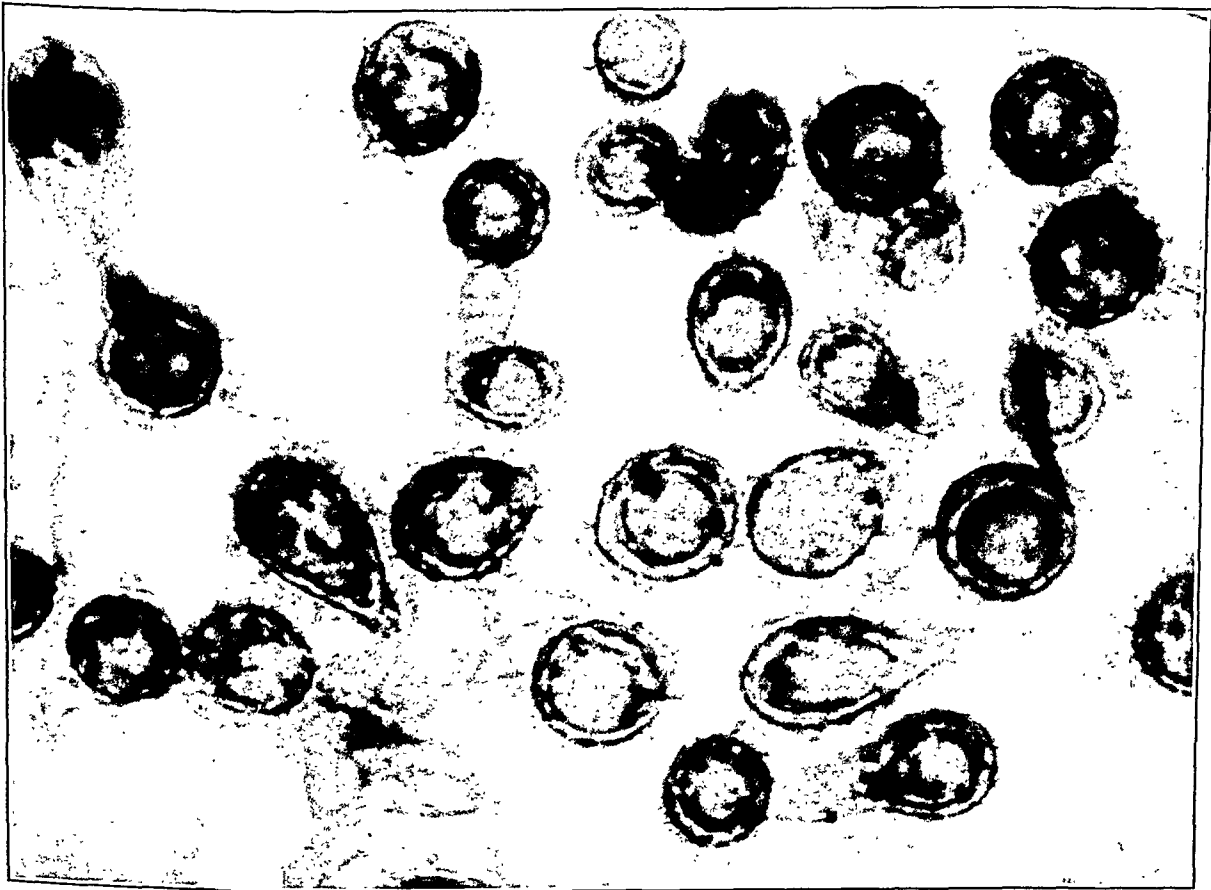
PLATE 159

FIG. 4. Photomicrograph of the yeast-like organism in the large mononuclear cells in the corium of the skin. $\times 1200$.

FIG. 5. Photomicrograph showing the large, thick-walled, spiculated chlamydospores which are so characteristic of the organism upon artificial culture medium. $\times 1200$.



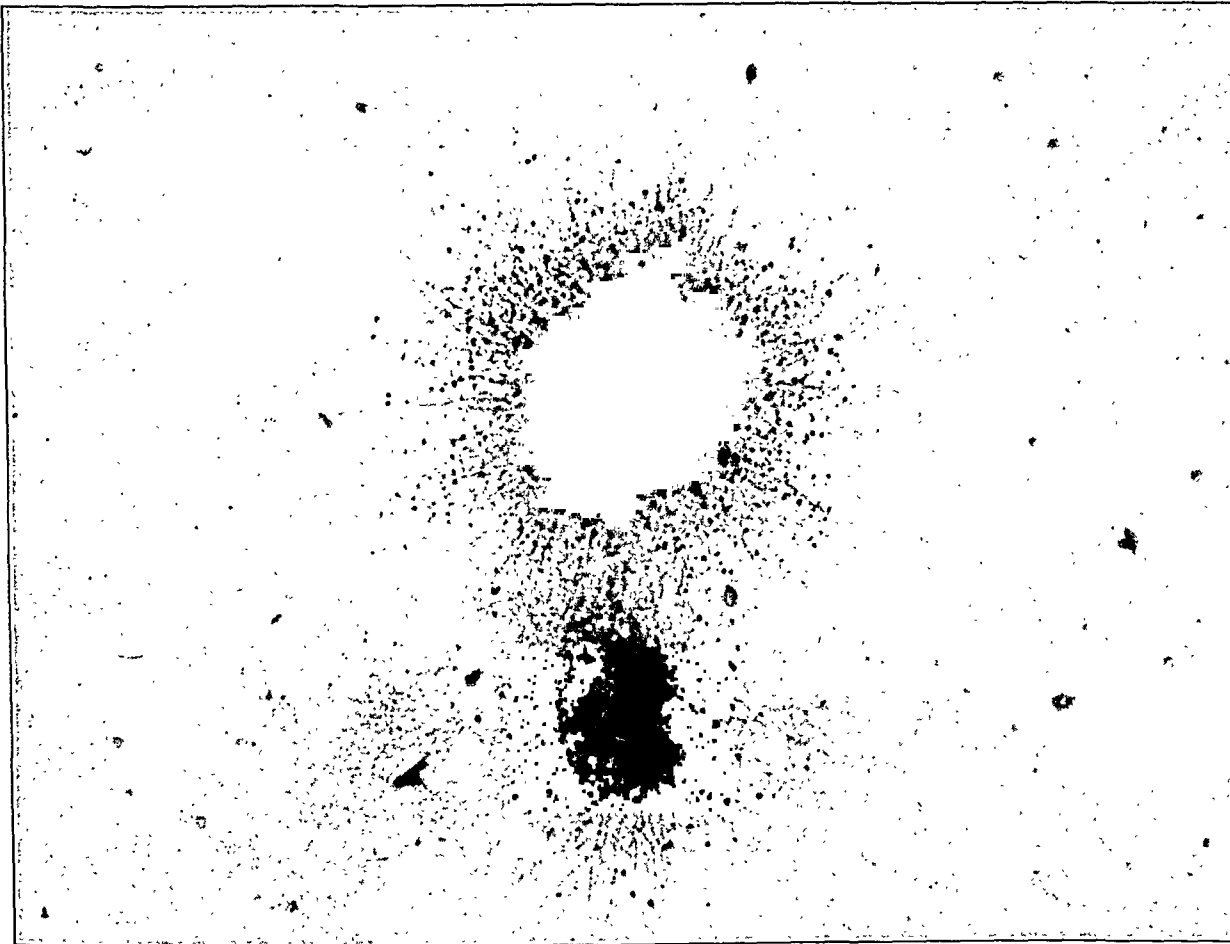
4



5

PLATE 160

- FIG. 6. Photomicrograph of a thallus of the organism grown on a glass slide. Note the large spores near the center of the thallus and the delicate, tangled mycelium. $\times 60$.
- FIG. 7. Photomicrograph of the adrenal of the human showing an area of caseation necrosis in which many organisms were found. $\times 70$.



6

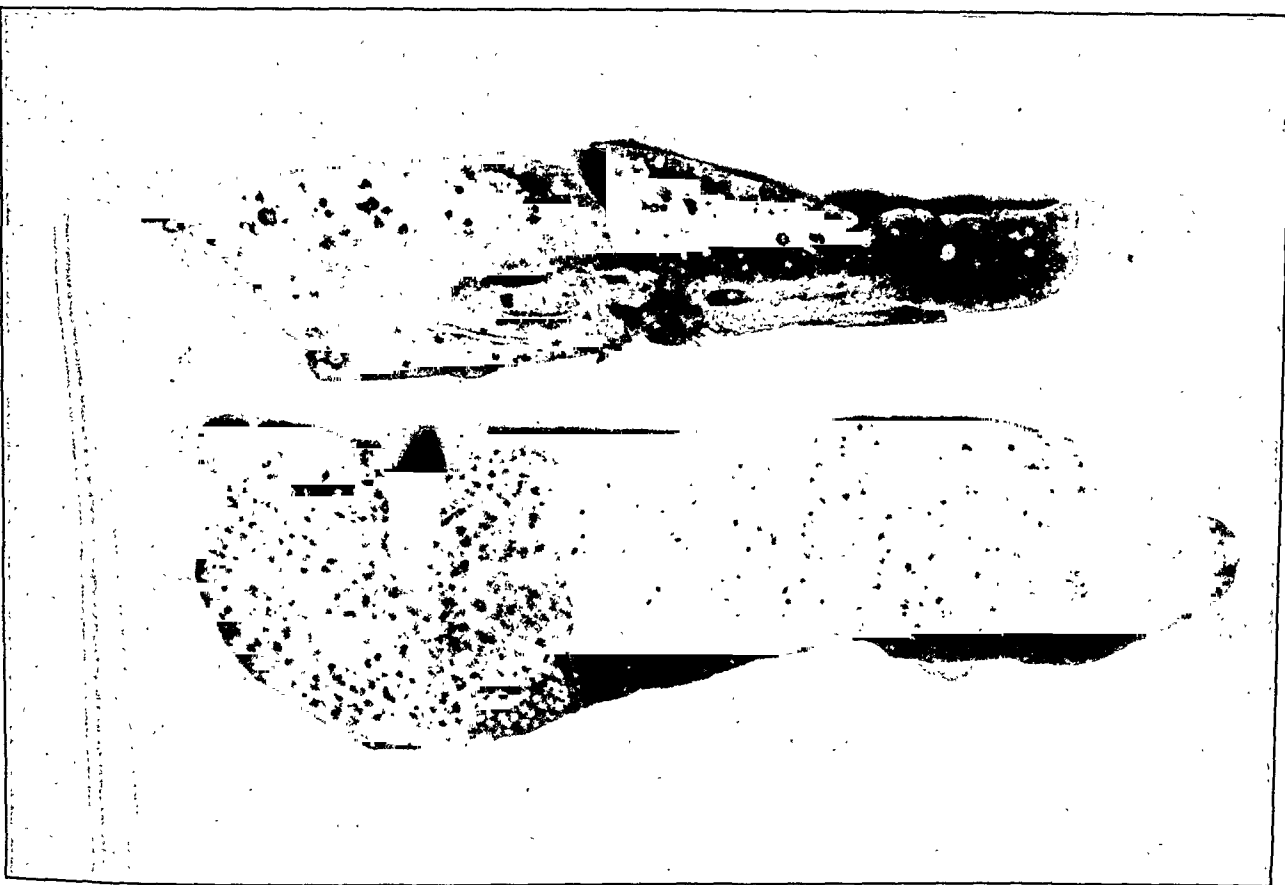


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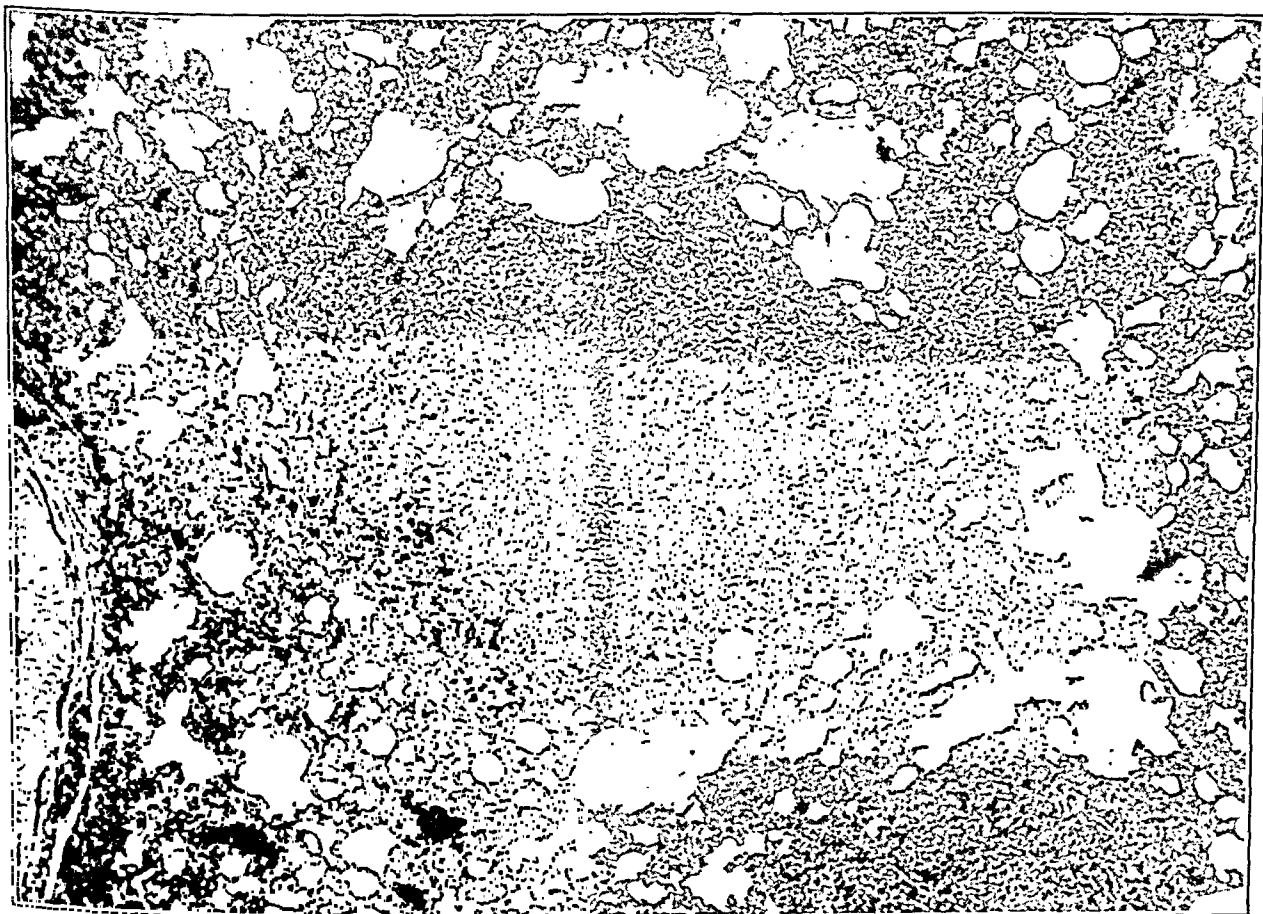
PLATE 161

FIG. 8. Photograph of the lesions in the lung and the spleen of the dog inoculated with the organism.

FIG. 9. Photomicrograph of the lung of a dog showing a granulomatous lesion of the disease. $\times 60$.



8



9

A FREE GROWTH PERIOD OF TUBERCLE BACILLI IN THE GUINEA PIG OMENTUM AS RELATED TO THE HYPERSENSITIVE STATE *

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The study of immune processes in tuberculosis is complicated by the fact that true immunity, in the sense in which that term is used in diphtheria or smallpox, does not exist. The patient who, to all clinical appearances, has completely recovered from tuberculosis may suffer a relapse, just as the experimental animal which has been "immunized" may be fatally reinfected with a large enough dose of virulent tubercle bacilli. At the same time, as was first adequately demonstrated by Römer,¹ something happens, in the laboratory animal and, presumably, in man, after an initial tuberculous infection, which renders the individual or the animal somewhat more resistant to reinfection. Just what changes occur in the body cells and fluids following this initial sensitizing infection is not clear.

A useful approach to the problem of immunity in tuberculosis and one which has been employed by numerous investigators, is the attempt to discover how the tubercle bacillus itself, with its resistant, waxy structure, is affected by inoculation in the normal and the "immune" animal. Thus Markl,² Kraus and Hofer,³ Manwaring and Bronfenbrenner,⁴ Bergel,⁵ Rist, Léon-Kindberg and Rolland⁶ and Dworski, Smith and Gardner⁷ all have studied peritoneal fluid withdrawn at intervals after intraperitoneal inoculation of the experimental animal, and Paterson⁸ has made a similar study of pleural exudates. All of these studies, however, are subject to the limitations imposed by the use of inflammatory exudates, most of the investigators having noted the early appearance of so-called Much's granules and the complete disappearance of acid-fast bacilli from the fluid of the respective serous cavities within 4 or 5 days after inoculation. In every case the bacilli were found to have disappeared more quickly in the reinoculated than in the normal animal.

* Received for publication July 9, 1934.

In the study which follows use has been made of the well known fact that bacteria, when inoculated in the peritoneal cavity, accumulate in large part in the omentum. Spread preparations of the omentum have been employed, a simple technic having been developed for staining such preparations. This use of omental spreads has made it possible to study the inoculated bacilli in their normal relation to the fixed tissue cells and the developing tubercles, a study that cannot be made adequately either with histological sections or with smears of the body fluids.

TECHNIC

Albino guinea pigs were employed in the major part of the experiments, since the reaction of this animal to tuberculous infection seems to approximate so closely that of man. In general, young animals from 200 to 300 gm. in weight were used, as the omentums of old guinea pigs contain too much perivascular fat to make good spread preparations.

In most of the experiments the well known H-37 human strain of *Mycobacterium tuberculosis* was used for the inoculum. A portion of the pellicle of a glycerine broth culture was weighed, after blotting off the excess fluid on sterile filter paper, and carefully ground with mortar and pestle in a few drops of sterile saline. Saline was then slowly added to give the dilution which was desired, usually 1 mg. of the culture per cubic centimeter of fluid.

Anyone who has attempted to prepare inoculums from cultures of the tubercle bacillus has experienced the difficulty of getting a uniform suspension of the organisms. Even after the most painstaking grinding relatively large clumps of bacilli remain in the triturated material. In the present experiments no attempt was made to remove these clumps by filtration since it was desired to maintain the known weight of inoculum. In most of the experiments the animals were given an amount of inoculum equivalent to 0.1 mg. of bacilli per 100 gm. of body weight, though in some series as much as five times this amount was employed. Whenever inoculums containing clumps of bacilli were employed, it was found important to take into the syringe sufficient inoculating fluid for only one animal at a time. If enough fluid for several animals is taken into the syringe the clumps of bacilli gravitate so rapidly that an uneven dose for the different animals results.

Another pitfall which must be guarded against in making intraperitoneal inoculations is that of losing all or a portion of the inoculum either by penetrating the bowel or by failing to enter the peritoneal cavity at all. In the present study the animals, with abdomen shaved, were lightly etherized and an incision 3 to 4 mm. in length made through the skin with scissors. By making use of this incision one could usually be certain of entering the peritoneal cavity with the inoculating needle. The best protection against penetration of the gut we have found to be an initial inoculation of 10 cc. of air with a separate syringe. If the air enters the peritoneal cavity the anterior abdominal wall will be bulged up uniformly, while if the air is forced into the appendix or some other portion of the gut a serpentine bulge is formed. Once the anterior abdominal wall has been separated from the underlying intestines by a layer of air the inoculating needle can be introduced with little fear of penetrating the bowel. If the inoculating dose should be lost in its entirety into the gut the omentum remains completely normal.

OMENTAL SPREADS

In order to follow the omental changes animals were killed at daily, or more frequent, intervals. During the first few days after inoculation omental spreads are easily made. After the first week, however, there is an increasing tendency for the free edges of the omentum to become matted together. Under these circumstances it is well to pull with forceps on the matted portion of omentum before it is removed from the animal, thus breaking most of the fibrinous adhesions. That part of the omentum which shows the maximum involvement is then freed with scissors and mounted on a clean glass slide, either directly or after first floating the tissue in a jar of normal saline. Using a pointed glass rod for manipulating the moist preparation, a single, thin layer of the omentum is isolated and stretched out from the major omental mass. By allowing this thin film of tissue to dry on the slide one obtains a point of attachment and can pull from this point on the main mass of omentum further to break apart its fibrinous adhesions. Finally one obtains a layer of omentum not more than one cell thick through most of its extent. The preparation is then allowed to dry in the air, after which it will be found firmly fixed to the slide. The steaming with carbol fuchsin completes the fixing process.

With minor exceptions the ordinary Ziehl-Neelsen technic for staining is employed. The preparation, flooded with carbol fuchsin, is heated to the steaming point on an aluminum plate, washed in tap water, flooded with acid alcohol, washed, and then immersed for 10 minutes in acid alcohol (3 per cent HCl in 95 per cent alcohol) in an attempt to decolorize the thicker portions of the preparation. After again washing in tap water the omentum is counterstained with methylene blue, washed and blotted. The preparation should then be allowed to dry for an hour at 37°C or for 12 hours at room temperature to allow the perivascular fat to "sweat out." Finally, any remaining portions which are too thick should be removed. The process is completed by merely immersing the dry preparation in absolute alcohol, then in xylol, and mounting in balsam. The result is a permanent preparation, easily and quickly available, which shows the entire developing tubercle with its content of bacilli and its surrounding cells. Such a preparation may be studied through most of its extent with the oil immersion lens.

RESULTS

I. Inoculation of the Normal Guinea Pig

The results which we have obtained in the early hours after intraperitoneal inoculation corroborate those reported by other workers. There is an initial acute inflammatory reaction, the tubercle bacillus being taken up first by polymorphonuclear cells. One of the surprising findings was the rapid disappearance of the large clumps of bacilli (Fig. 1) which were included in the inoculum. A few hours after inoculation these clumps had been almost completely broken up by the polymorphonuclears, each cell taking up from one to a dozen bacilli (Figs. 2 and 3), and by the end of 24 hours the clumps had completely disappeared. At 2 hours there are relatively few large mononuclear leukocytes, but an occasional one will be found which has phagocytosed bacilli. In no case, however, have we found the mononuclear cells phagocytosing polymorphonuclears at this stage. Unfortunately, the Ziehl-Neelsen stain used for our preparations did not enable us to differentiate between polymorphonuclear neutrophils and eosinophiles, the latter, according to Dworski, Smith and Gardner,⁷ being the cell most actively phagocytic for the tubercle bacillus in the first few minutes after inoculation.

After 24 hours the peritoneal fluid and the omentum still show numerous polymorphonuclear leukocytes, but very few of these cells contain tubercle bacilli. Many of the polymorphonuclears which phagocytosed bacilli during the first few hours have now been taken up by large mononuclear cells (Figs. 4 and 5).^{*} The bacilli, as seen after this double phagocytosis, exhibit marked irregularities in staining and in many cells non-acid-fast granules may be seen which probably represent the so-called Much's granules. Also, occasional mononuclear cells are seen which have evidently taken up bacilli directly (Fig. 6). It will be noted that these bacilli appear to lie within cytoplasmic vacuoles. Furthermore, they appear better preserved than the bacilli in Figures 4 and 5 which were first phagocytosed by polymorphonuclears.

While many damaged polymorphonuclear leukocytes are taken up by large mononuclear cells, as illustrated in Figures 4 and 5, a large portion of the polymorphonuclears bearing tubercle bacilli apparently gravitates to the milk spots (*tache laiteuse*) of the omentum or to the perivascular fat tissue. The dark areas shown about the blood vessel in Figure 7 are made up almost entirely of polymorphonuclears which have encroached upon the perivascular fat cells. Many of these polymorphonuclears still carry their burden of bacilli. Within 3 days after inoculation the perivascular areas have become a dense cellular mass composed largely of mononuclear cells (Fig. 8). It is the opinion of Gardner,¹⁰ who has studied this phenomenon in regular histological sections, that the mononuclear cells proliferate *in situ* to form the tubercle-like nodules about the blood vessels. It is in these areas that caseation first appears.

The Appearance of Freely Growing Bacilli: Gardner¹¹ has pointed out the remarkable clearing of the acute inflammatory reaction within 3 days after intraperitoneal inoculation of the normal guinea pig with tubercle bacilli. We have observed the same thing in spread preparations of the omentum. At 4 days the preparations show the dense perivascular infiltration, the enlarged milk spots and occasional clumps of mononuclear cells which apparently proliferate *in situ* on the surface of the omentum to form miliary tubercles (Fig. 9). At 4 days, also, a phenomenon which we have not hitherto

^{*} A recent paper by Vorwald⁹ indicates a similar transference of bacillus-containing polymorphonuclears to the large mononuclear cells in the lung of the rabbit after intravenous inoculation with H-37.

seen reported makes its appearance. In association with certain cells may be seen masses of bacilli growing in parallel strands just as they might grow on the surface of glycerine broth (Figs. 10 and 11). These masses may be immediately adjacent to, or some little distance from, the groups of cells that are forming tubercles (Figs. 10, 11, 12 and 13) and, surprisingly enough, show no sign whatever at this stage of attracting inflammatory cells of any type (Figs. 14 and 15). The bacilli commonly follow the cytoplasmic outline of the cell with which they are associated and at times are looped about the cell nucleus (Fig. 16). However, they appear to be growing on the surface of, rather than within, the cytoplasm. From all appearances the cells in which, or on which, the bacilli are growing are the ordinary ones which make up the major portion of the omental network (Figs. 17 and 18). They show no deleterious effect from the symbiotic growth. These organisms, because of the capacity which they exhibit for growth without the least interference from either the cells or body fluids of the animal in which they are proliferating, have been termed *freely growing bacilli*.

That the microorganisms which have just been described have actually proliferated and do not represent merely clumps of bacilli from the inoculum is indicated, first, by the fact that no such clumps are found in the omentum in the interim between inoculation and the 4th day and that all large clumps have disappeared from the peritoneal fluid by the end of 24 hours; secondly by the fact that the bacilli are in parallel strands just as they grow on culture media, while the inoculated bacilli, after phagocytosis by either polymorphonuclears or monocytes, are found helter-skelter in the cell without any regular arrangement. Thirdly, only a few small colonies of freely growing bacilli are found in an occasional omentum at the 4th day, while, on the 6th day the colonies are larger and more numerous and are found with uniformity in the omenta of most of the animals. Fourthly, the freely growing forms do not appear in the omentum of "immunized" or secondarily inoculated guinea pigs. Finally, various control inoculations (to be described in detail later) made with caseous material, heat-killed bacilli, and timothy grass bacilli prove that the tubercle bacillus does actually proliferate in the animal body at a certain period after inoculation without any inflammatory response on the part of the diseased animal. The great regularity with which the free proliferation of tubercle bacilli

occurs in the body of the normal guinea pig is indicated by the accompanying table.

TABLE I

*Normal Guinea Pigs Inoculated with H-37 from Glycerine Broth Cultures **

Days after inoculation	1	2	3	4	5	6	7	8	9	10	11	12	13-78
Total No. pigs sacrificed	24	13	12	15	15	26	18	18	14	11	13	12	103
No. of pigs <i>positive</i> for free bacilli ..	0	0	0	6	11	21	16	6	2	1	1	0	0
No. of pigs <i>negative</i> for free bacilli ..	24	13	12	9	4	5	2	12	12	10	12	12	103

* Based upon 21 series of guinea pig inoculations.

As indicated, the freely growing forms were found first after an interval of 4 days. Four days is about the length of time required for the H-37 strain to establish itself on favorable culture mediums and this fact probably explains why freely growing bacilli were not found before the 4th day after inoculation. From the 4th day the number of animals showing the freely growing bacilli rises to 81 per cent at 6 days* and 89 per cent at 7 days, falling off abruptly after the 7th day.

The Use of Caseous Material as Inoculum: Five series of animals were inoculated with caseous material obtained from cold abscesses, the latter being the result of inoculating guinea pigs subcutaneously with H-37. This caseous material can be readily triturated and diluted with saline to give a uniform suspension. The amount of inoculum used varied from 10 mg. of caseous material per pig in 1 series to a maximum of 50 mg. per pig in another series. Smears of the inoculum showed only scattered acid-fast bacilli, while in 2 series the caseous material contained no demonstrable acid-fast organisms whatsoever. In every series, however, masses of freely growing tubercle bacilli made their appearance, Table II presenting the accumulated data from the 5 series.

* A careful reading of one of Krause's¹² early papers indicates that he must have observed freely growing bacilli in the iliac lymph nodes of guinea pigs after subcutaneous inoculation in the groin. In describing the masses of bacilli which have proliferated in these nodes at 6 days Krause says, "In none of these particular fields did the bacilli appear to lie within the cells. . . ."

It will be noted from Table II that the freely growing bacilli in these series were somewhat more tardy in making their appearance than when the inoculum was obtained from broth cultures. When they eventually appeared, however, the freely growing forms were perfectly typical in arrangement (Figs. 19 and 20).

TABLE II

*Normal Guinea Pigs Inoculated with H-37 Caseous Material obtained from Cold Abscesses **

Days after inoculation	1	2	3	4	5	6	7	8	9	10	11	12	13-47
Total No. of pigs sacrificed	4	2	3	2	3	2	3	1	6	1	1	1	47
No. of pigs <i>positive</i> for free bacilli . .	0	0	0	0	0	2	3	1	2	0	0	1	0
No. of pigs <i>negative</i> for free bacilli . .	4	2	3	2	3	0	0	0	4	1	1	0	47

* Based upon 5 series of guinea pig inoculations.

A single series of animals was inoculated with the bovine strain, H-61, and another series with caseous material obtained at the autopsy table from a case of generalized tuberculosis in man. In both series beautiful examples of freely growing bacilli were found.

The Use of Dead Bacilli and of M. Phlei as Inoculum: Three series of pigs were inoculated intraperitoneally with H-37 killed by boiling and 1 series with the same organisms killed by exposure to direct sunlight. In the 4 series 26 guinea pigs were employed. Tubercles were formed in the omentums of these guinea pigs and, in the tubercles, faintly staining acid-fast bacilli were found for as long as 22 days after inoculation. Though the inoculums — in each case 1 mg. per pig — contained large clumps of bacilli, in no instance was anything found in the omentum suggestive of the freely growing form of the bacillus.

In 4 series, utilizing 33 pigs, the timothy grass bacillus, *M. phlei*, was employed as inoculum. The relatively small lipid content of this bacillus, as compared with the pathogenic acid-fast strains, has been pointed out by Anderson,¹³ a fact that may explain the early disappearance of the inoculated grass bacilli from the omental preparations. However, tubercles are formed sooner than after in-

oculation with H-37 (Figs. 21 and 22). Acid-fast bacilli can be found in these tubercles in large numbers during the first 24 hours, but by 3 days and thereafter bacilli can no longer be found. The tubercles do not progress to caseation.

II. The Inoculation of Hypersensitive Guinea Pigs and of Normal Rabbits

The results from the inoculation of hypersensitive pigs contrast strikingly with those obtained from the normal animals. In 3 series the pigs were given a subcutaneous sensitizing dose of H-37 from 3 to 5 weeks before the secondary intraperitoneal inoculation. As far as the exudative reaction in the hypersensitive animal is concerned our findings again corroborate those of Gardner.¹¹ The initial outpouring of polymorphonuclears is greater than in the normal animal. Tubercles form more rapidly, and the omentum, within 4 days after the second inoculation, is matted together in a dense sausage-shaped mass which cannot be separated without tearing the tissues. Within this matted omentum may be found accumulations of creamy caseo-pus, while a dense fibrinopurulent exudate is frequently found adhering to the surfaces or edges of the liver and spleen. If, at daily intervals, smears are made of either the pus or the fibrinopurulent exudate they will be seen to contain the same large clumps of acid-fast bacilli that were included in the original inoculum (Fig. 23). These clumps are closely surrounded by polymorphonuclear leukocytes, the cells being literally plastered against the edges of the bacillary mass. An occasional cell in the smear (either polymorphonuclear or mononuclear) may contain a few bacilli, but there is no sign of the breaking up of bacillary clumps through the phagocytosis which occurs in the normal pig.

At 10 days the clumps are still found, somewhat more rounded or oval in shape, with a ring of polymorphonuclears still pressing tightly against the bacilli (Figs. 24, 25 and 26). The latter appear to have undergone partial lysis, no longer staining as sharply as in the early days after inoculation. Thus, in the hypersensitive guinea pig the clumps of bacilli which may be included in the inoculum are not broken up by phagocytosis, as they are in the normal animal. Furthermore, at no time in the hypersensitive animals is the phenomenon of free proliferation of the inoculated tubercle bacilli ob-

served. In none of the 31 animals used in the 3 series was there any suggestion of that free growth of tubercle bacilli which occurs in the normal guinea pig.

Since the normal rabbit has a high degree of natural resistance to infection with the human type of tubercle bacillus, it was decided to inoculate some of these animals. Accordingly, a small group, 9 rabbits in all, was inoculated intraperitoneally with H-37 on the same basis as the guinea pigs, namely, 0.1 mg. of bacilli per 100 gm. of body weight. None of the inoculated rabbits showed at any time any free growth of tubercle bacilli.

III. The Fate of the Freely Growing Bacilli

Up to 6 days after inoculation the freely growing bacilli show no evidence of attracting phagocytic cells (Figs. 10 to 18), although by the 7th day they usually begin to exert a chemotactic influence upon the polymorphonuclear leukocytes. Frequently in a single omental preparation at this stage one finds a few phagocytic cells hovering about at a little distance from the bacilli (Figs. 27 and 28), while another colony of bacilli may have a polymorphonuclear directly applied to it (Fig. 29). Still another colony may be completely broken up by the invasion of polymorphonuclear leukocytes (Figs. 30 and 31). The bacilli at this period show a marked degree of beading (Fig. 32).

At 7 days, then, the omentum of a single animal may show considerable variation in the status of the masses of freely growing bacilli, though by the 8th day the freely growing forms have disappeared in the majority of the animals. In their place we find evidence of the formation of a secondary series of tubercles, and by 9 days this phenomenon is well established. At this time the omental preparations show two definite vintages, so to speak, of tubercles — large ones which have formed about the original bacilli of the inoculum, and minute ones which are in the process of forming about the once freely growing bacilli (Fig. 33). It is frequently impossible to make out the bacillary content of the large tubercles at this stage, even when they are teased apart, while in the minute tubercles bacilli are clearly visible (Fig. 34).

During the period from 10 to 14 days after inoculation one gains the impression that the number of acid-fast bacilli is definitely less,

occasional preparations showing beaded, non-acid-fast forms. Acid-fast bacilli, if found at this stage, are frequently bizarre in shape, as shown in Figure 35. At much later stages the omentum still shows acid-fast bacilli which are irregular in length and in staining reaction (Fig. 36). Some of these bacilli appear to lie free on the surface of cells, but there are no freely growing colonies such as characterize the 4 to 7 day interval. Any phagocytosis at this stage is apparently by cells of the mononuclear series (Fig. 36).

DISCUSSION

From the data presented it is evident that tubercle bacilli of the H-37 strain, after intraperitoneal inoculation in the normal guinea pig, pass through certain definite changes, as far as their chemotactic relation to cells of the host animal is concerned. The inoculated bacilli attract and are taken up by polymorphonuclear leukocytes, which then either move to the perivascular tissues or milk spots, or are phagocytosed in turn by large mononuclear cells. At 4 to 6 days the freely growing forms appear and show no sign of chemotactic influence until the 7th day, when they again attract the polymorphonuclear cells. It seems probable that this variable picture, as regards chemotaxis, is due to one of two things — either to changes that occur in the cells and (or) fluids of the infected animal, or to changes that occur in the biochemistry of the inoculated microorganisms. In favor of the former hypothesis is the fact that hypersensitiveness, as indicated by a positive tuberculin reaction, appears within 6 to 12 days after experimental inoculation of the guinea pig with tubercle bacilli.¹⁴ To determine whether there is any correlation between the appearance of a positive tuberculin reaction and the disappearance of freely growing bacilli in a given animal, a small series of pigs was tested by the Mantoux method. In this small series there was no absolute correlation observable, the freely growing bacilli having usually disappeared several days before the appearance of a positive tuberculin reaction. It was noted, however, that whenever the tuberculin test did become positive in an animal there was no longer any sign of freely growing bacilli in that animal's omentum.

In favor of the hypothesis that some change in the biochemistry of the bacillus is responsible for the chemotactic cycle is the abrupt

falling off in the percentage of animals positive for freely growing bacilli.

As indicated in the curve below, the proportion of animals showing the freely growing forms falls from 89 per cent at 7 days to 9 per cent at 10 days, a rather more abrupt drop than one would expect if the decrease were due merely to the gradual development of hypersensitiveness in the animals during the interval from 6 to 12 days. The abrupt appearance of freely growing bacilli at 4 days we have attributed to the latent period required for the H-37 strain to adjust

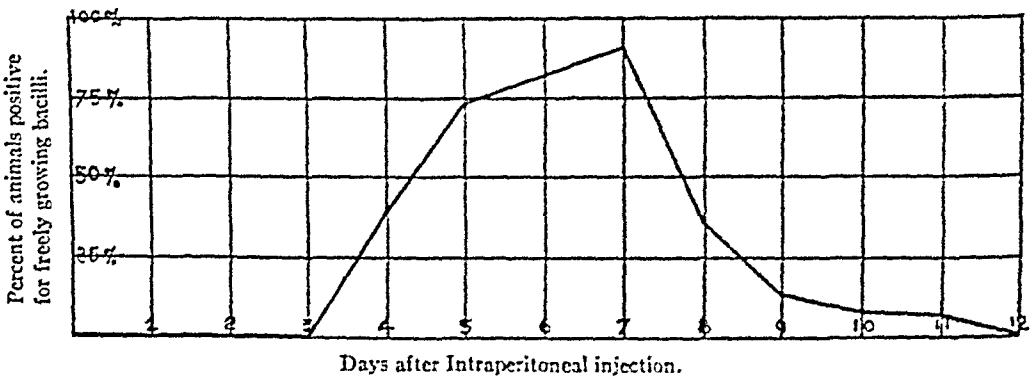


CHART I

itself to culture mediums of any type. Possibly the abrupt disappearance of these freely growing forms is again dependent upon some change in the characteristics of the bacilli themselves. Kahn¹⁵ has demonstrated in beautiful manner that the H-37 strain of *M. tuberculosis* may pass through a complicated pleomorphic cycle when grown on certain culture mediums. The marked beading of the freely growing bacilli (Figs. 29, 30 and 32) is suggestive of the zoning within certain bacilli, which Kahn describes as occurring in his microdroplet cultures, or of the granules to which Gróh¹⁶ attaches such significance.

It is impossible, at the present time, to decide definitely whether the abrupt reappearance of positive chemotaxis and the concomitant disappearance of the freely growing bacilli is due to the development of immune bodies in the infected animal or to changes in the bacillus itself, or to both. It seems certain, however, from our experience with the 31 secondarily inoculated animals, that the phase of free growth does not occur at all in an animal once it has been rendered hypersensitive. Thus, whatever else it may mean,

the positive tuberculin reaction in a guinea pig indicates that that animal is no longer susceptible to the type of free, unencumbered growth of tubercle bacilli, which takes place with uniformity in the normal animal.

Since we have noted that the freely growing bacilli disappear *before* the tuberculin test becomes positive, it seems possible, if not probable, that some reaction more delicate than the tuberculin test, which will indicate this changed condition of the animal, may be discovered. A further basis for this hypothesis is to be found in our results with the series of normal rabbits which failed to show free growth of the inoculated tubercle bacilli at any time. The normal rabbit does not, of course, exhibit a positive tuberculin reaction. It is interesting to speculate upon the possibility that the natural resistance of rabbits to infection with the human type of *M. tuberculosis* is related in some way to this failure of the free growth of the inoculated bacilli.

In the occurrence of the free growth of tubercle bacilli within the body of the normal guinea pig and the failure of that type of growth to occur in the hypersensitive animal we find the refutation of those workers who maintain that the difference between the reactions of the two types of animals is merely quantitative.¹⁷ To be sure, the inflammatory reaction in the hypersensitive animal is quantitatively greater at the outset, but this reaction is maintained, instead of clearing up completely, as in the normal pig. This difference seems to us qualitative in nature, particularly when viewed in the light of the different effect upon the growth of the tubercle bacillus during the first week after inoculation. Rich,¹⁸ in arguing against the acute inflammatory reaction of the allergic animal as a factor in "localization" of injected bacilli says: "Certainly, we ourselves have never been able to discover any difference between the number of stainable bacilli at the site of inoculation into the skins of normal and immune animals during the first few days after the inoculation; and after that, the number of bacilli in the area in the normal animals surpasses that in the immune animals — quite the reverse of what one would expect to find if the bacilli were quickly drained from the site in the normal animals, and actually held there bodily for days in the immune animals." Though the given data, particularly with regard to time intervals, are incomplete, it is our impression that Rich, in finding an increased number of organisms in his normal guinea pigs

after several days, was dealing with freely growing forms of the tubercle bacillus. In a series of normal animals which we inoculated subcutaneously, evidence was obtained that the free growth of tubercle bacilli occurs 6 or 7 days after subcutaneous inoculation, as well as after the intraperitoneal route of infection.*

An impression which inevitably follows working with omental preparations from several hundred infected guinea pigs is that one must have a very definite respect for the function of the polymorphonuclear leukocyte in the immune processes of tuberculosis. The polymorphonuclear has fallen into low esteem in this disease due, in part, to the well known fact that the opsonic index is lower in the animal rendered hypersensitive to tuberculosis than in the normal animal. To Zinsser¹⁹ “. . . it is perfectly plain at the present time that polymorphonuclear phagocytosis has no protective value in tubercle bacillus infection. Indeed the tubercle bacilli are carried by the polynuclears throughout the body and any intra-cellular destruction that takes place is the function of clasmatocytes and giant cells.”

While it is undoubtedly true that tubercle bacilli are disseminated by the polymorphonuclear leukocytes following a primary infection, it is also evident that the bacilli grow at a certain period in perfect symbiosis with the omental cells — cells of the connective tissue series which are, presumably, closely related to the clasmatocytes. Furthermore, it is the polymorphonuclears that first attack and break up the freely growing bacilli and initiate the process of renewed tubercle formation. Also, it is the polymorphonuclears that we find, in the hypersensitive pigs, plastered about the clumps of inoculated bacilli — not phagocytosing them but preventing the clump from breaking up, helping to localize the bacilli, as Krause and Willis²⁰ have shown and, we surmise, helping to prevent the free growth which occurs in the normal animal. This reaction of the polymorphonuclear cells in the hypersensitive animal is perfectly compatible with the existence of a low opsonic index, the low index, in fact, being an indication of the new function of the polymorphonuclear leukocyte in these animals, in which as Krause²¹ says, “An almost immediate inflammatory outpouring hems in the bacilli more

* The possibility that these freely growing bacilli may represent an R dissociant has been considered, though no actual experimental work bearing on this point has been performed.

or less effectively and thus delays or prevents their spread which is so facile and rapid in the non-tuberculous, non-allergic animal."

We can agree with Rich's¹⁸ conception of the local fixation of bacilli as a phenomenon "separate and dissociable" from allergy. At the same time, however, we believe that the polymorphonuclear leukocyte plays an important rôle in determining whether or not inoculated tubercle bacilli may grow freely for a certain period in the body of the host, and that this cell, therefore, is a factor which must be given due consideration in any discussion of immunity in tuberculosis.

SUMMARY

1. After intraperitoneal inoculation in the guinea pig the H-37 strain of tubercle bacillus is first subject to phagocytosis by polymorphonuclear leukocytes. Then certain of the bacilli grow freely for a period on, or in, the cells of the omentum without exhibiting any chemotactic influence. At the end of this period the bacilli again attract polymorphonuclear leukocytes.

2. In guinea pigs that have been rendered hypersensitive to tuberculosis, and in normal rabbits, free growth of inoculated tubercle bacilli does not occur.

3. The relation of free growth and of polymorphonuclear phagocytosis to resistance in tuberculosis is discussed.

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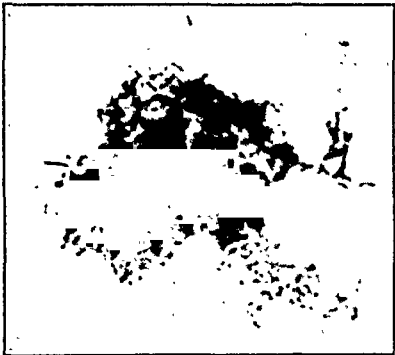
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DESCRIPTION OF PLATES

PLATE 162

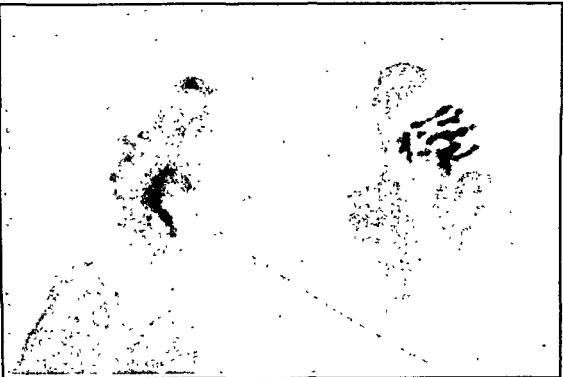
- FIG. 1. One of the large clumps of tubercle bacilli which characterized inoculum employed in most of the experiments. Photomicrograph taken with blue light. $\times 1500$.
- FIG. 2. Numerous polymorphonuclears in omental spread. Two hours after inoculation. Most of the cells have phagocytosed tubercle bacilli. Blue light. $\times 330$.
- FIG. 3. Polymorphonuclear cells with phagocytosed bacilli. From omental spread made 4 hours after inoculation. Blue light. $\times 1500$.
- FIG. 4. Large mononuclear cell showing phagocytosis of polymorphonuclears which had taken up bacilli. Found in smear of peritoneal fluid 24 hours after inoculation. Blue light. $\times 1500$.
- FIG. 5. Cell from same preparation as Fig. 4. Blue light. $\times 1500$.
- FIG. 6. Cell of mononuclear series which has phagocytosed bacilli directly. From omental spread made 48 hours after inoculation. Blue light. $\times 1800$.



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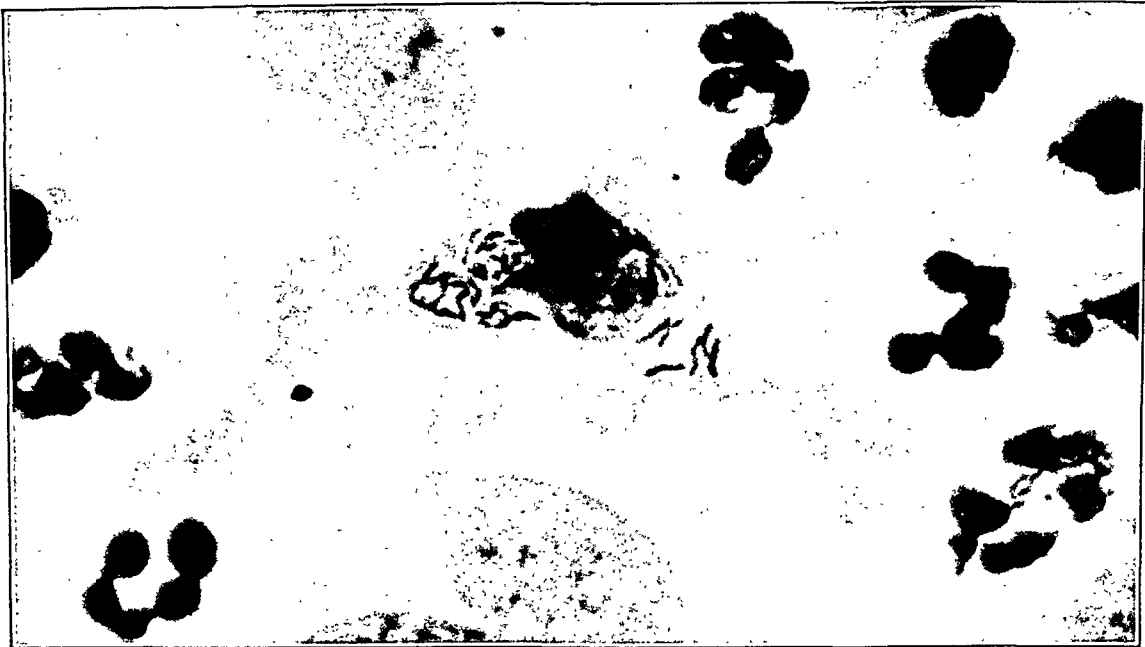
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PLATE 163

FIG. 7. The dark areas in the photograph are masses of polymorphonuclear leukocytes which have migrated to the perivascular fat tissue. From omental spread made 24 hours after inoculation. White light. $\times 25$.

FIG. 8. Omental spread made 3 days after inoculation. Large mononuclear cells replace the polymorphonuclears and almost completely obliterate the perivascular fat cells. Arrow points to a circle of cells formed as the result of the localization of a bubble of air introduced at time of intraperitoneal inoculation. White light. $\times 25$.

FIG. 9. Omental spread 4 days after inoculation showing clump of large mononuclear cells. These cells seem to proliferate locally, by amitotic division, to form miliary tubercles. Blue light. $\times 600$.

FIG. 10. More advanced stage of miliary tubercle formation, with freely growing bacilli on cell outlined in rectangle. Omental spread made 4 days after inoculation. Blue light. $\times 165$.

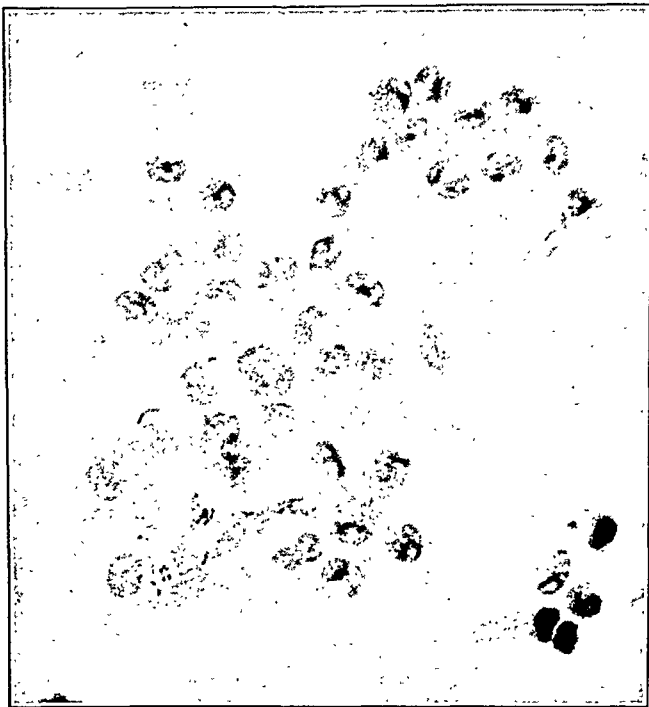
FIG. 11. Higher magnification of cell outlined in Fig. 10. Freely growing bacilli are clearly shown. Blue light. $\times 1500$.



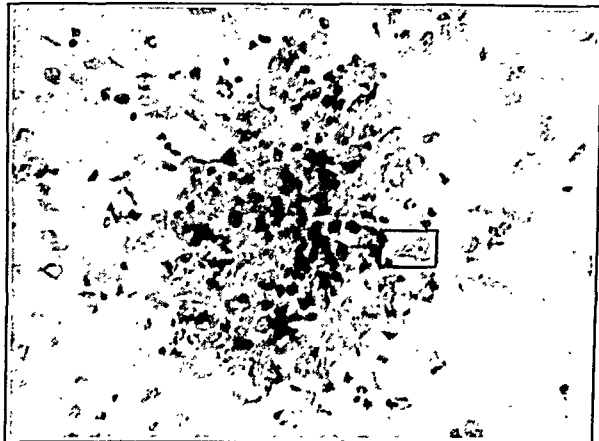
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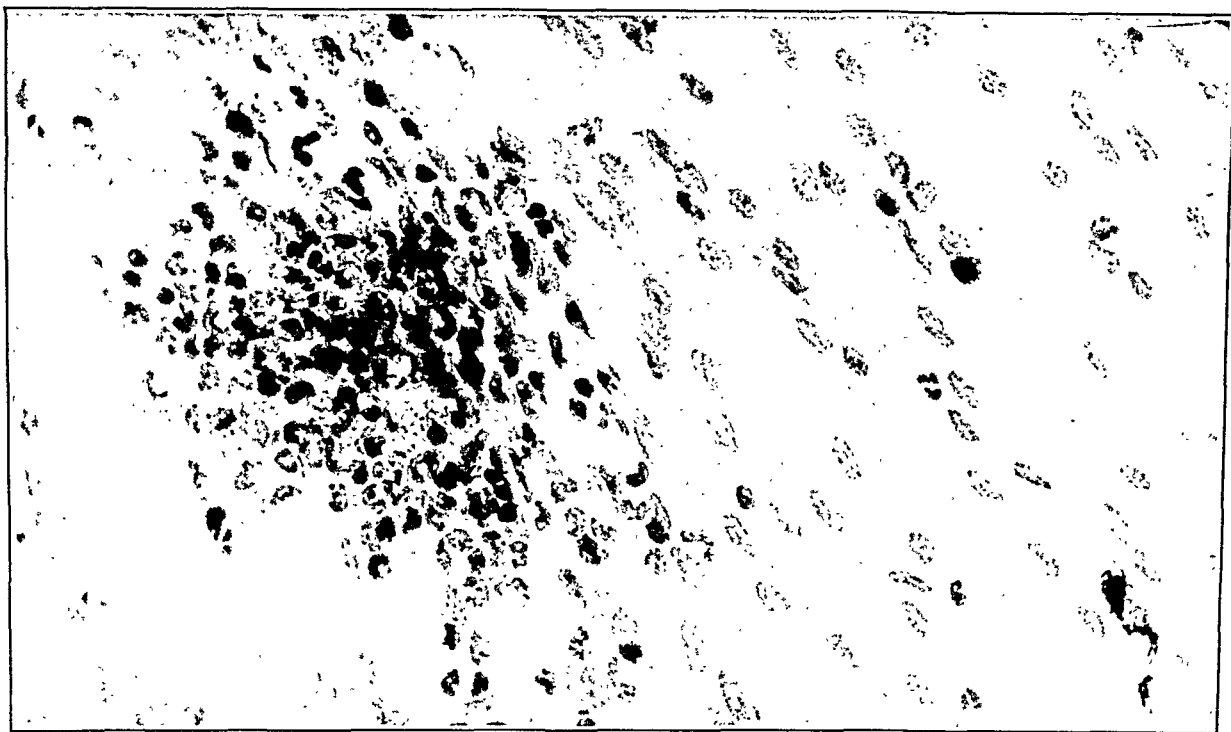
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PLATE 164

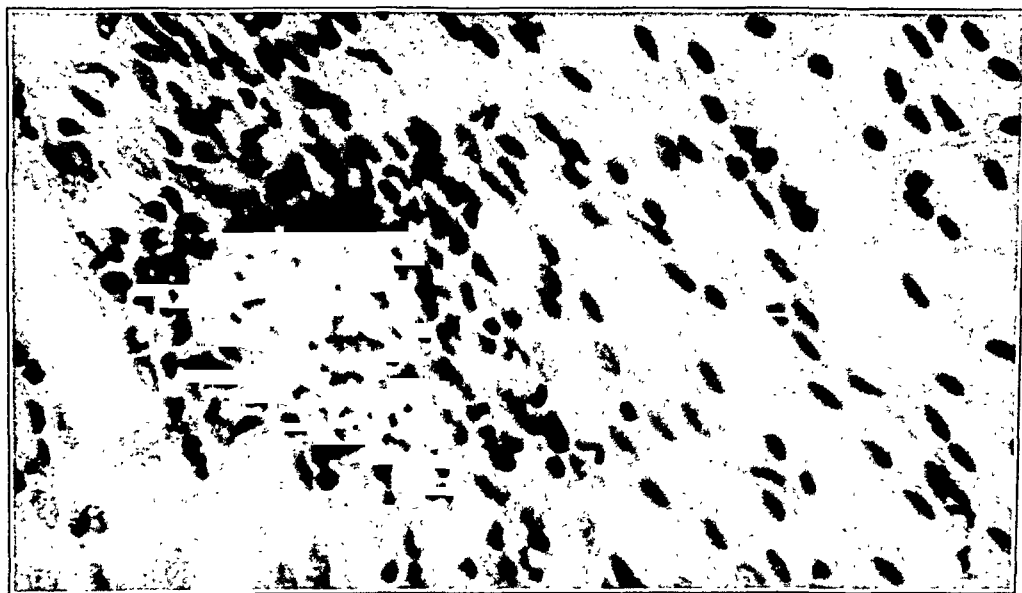
FIG. 12. Freely growing bacilli, in lower right hand corner, at some distance from a developing tubercle. Omental spread made 6 days after inoculation. Blue light. $\times 330$.

FIG. 13. Same tubercle and bacilli as shown in Fig. 12. Photomicrograph taken with green light to bring out the elastic network which characterizes the guinea pig's omentum. Green light. $\times 300$.

FIG. 14. Numerous colonies of freely growing bacilli on omental cells 6 days after inoculation. Blue light. $\times 600$.



12



13



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PLATE 165

FIG. 15. Freely growing bacilli on omental cells 6 days after inoculation. Blue light. $\times 1500$.

FIG. 16. Freely growing bacilli on omental cell 6 days after inoculation. Frequently the bacilli curve about the cell nucleus as shown. Blue light. $\times 1800$.

FIG. 17. Freely growing bacilli on omental cells 6 days after inoculation. To all appearances the affected cells are no different from the surrounding cells. Blue light. $\times 350$.

FIG. 18. Higher magnification of cells outlined in Fig. 17. Blue light. $\times 1800$.

FIG. 19. Freely growing bacilli at some distance from a large tubercle formed 7 days after inoculation with caseous material. Tubercle formation was more rapid in this series than after inoculation of H-37 from culture. Blue light. $\times 165$.

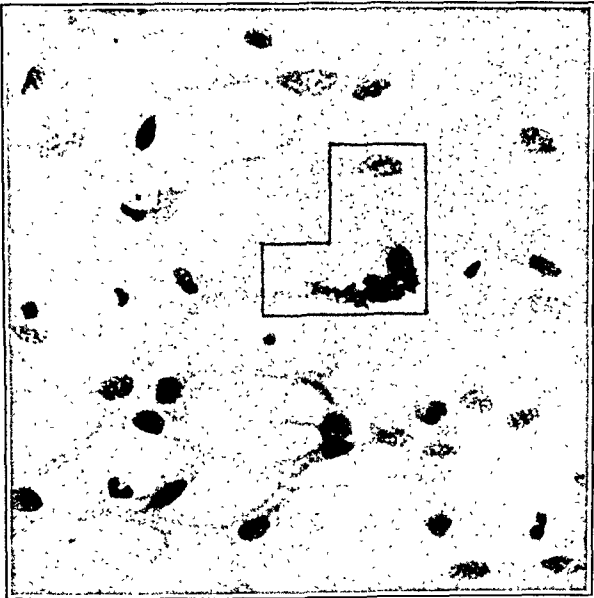
FIG. 20. Higher magnification of bacilli outlined in Fig. 19. Blue light. $\times 1500$.



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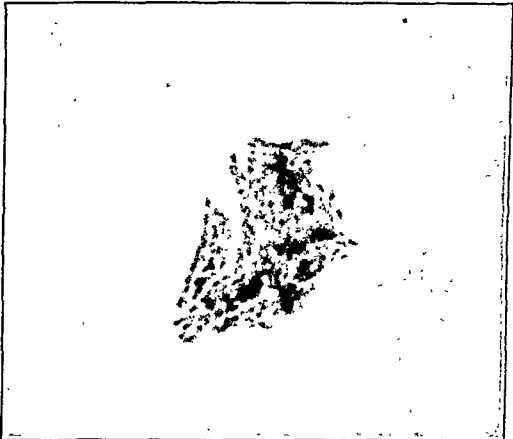
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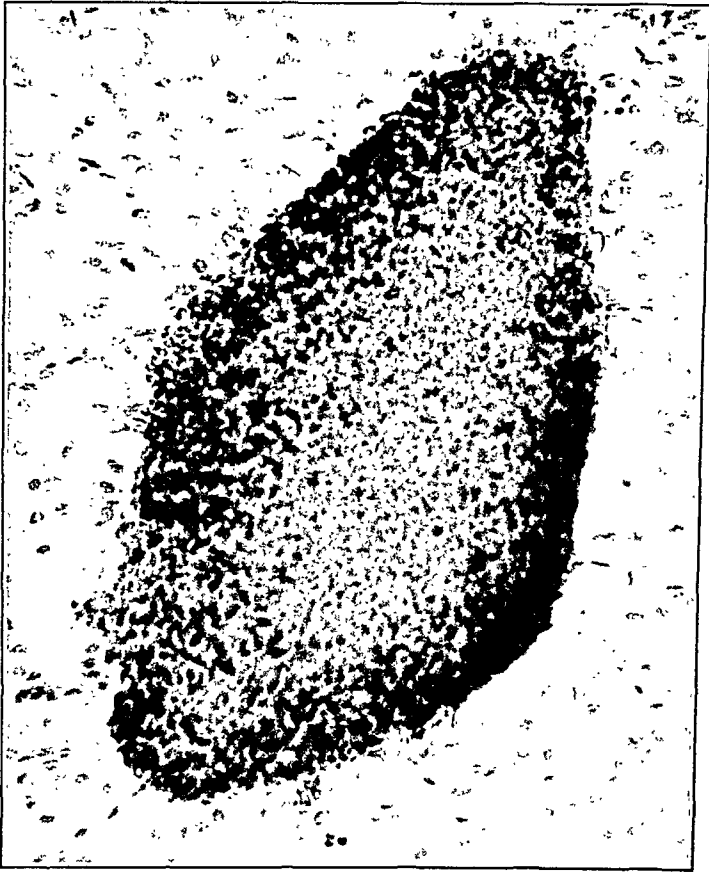
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PLATE 166

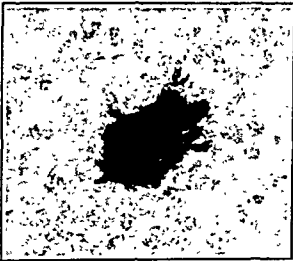
- FIG. 21. Tubercles in omental spread made 48 hours after inoculation with timothy grass bacilli. Tubercle formation in this series, too, proceeded more rapidly than after H-37 inoculation. White light. $\times 165$.
- FIG. 22. Tubercle 6 days after inoculation with timothy grass bacilli. White light. $\times 165$.
- FIG. 23. Omentum of hypersensitive guinea pig 48 hours after inoculation. A clump of bacilli from the original inoculum still exists. Blue light. $\times 330$.
- FIG. 24. Smear of caseo-pus found encapsulated in omentum of hypersensitive guinea pig 10 days after inoculation. The smear showed numerous clumps of bacilli closely surrounded by polymorphonuclear cells. Blue light. $\times 330$.
- FIG. 25. Same clump of bacilli as shown in Fig. 24. Photomicrograph taken with white light to bring out the polymorphonuclears. White light. $\times 1500$.
- FIG. 26. Same bacilli as in Fig. 25. Photomicrograph taken with blue light. $\times 1500$.



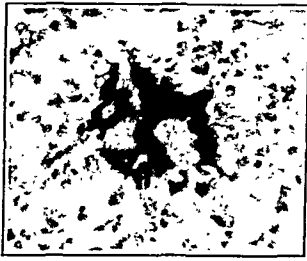
21



22



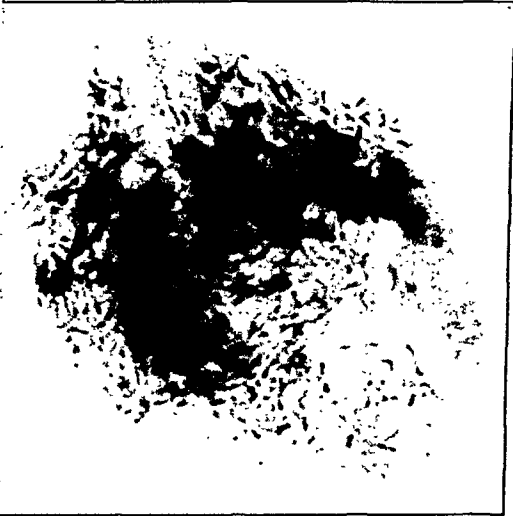
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PLATE 167

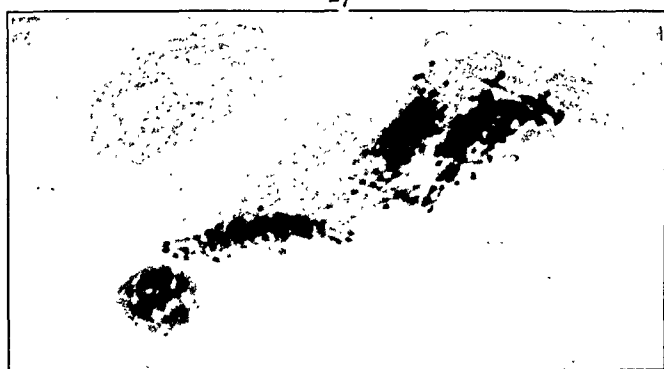
- FIG. 27. Freely growing bacilli with adjacent polymorphonuclear cells. Omental spread made 7 days after inoculation. Blue light. $\times 1500$.
- FIG. 28. Freely growing bacilli with adjacent polymorphonuclear cells. Seven days after inoculation. Blue light. $\times 1500$.
- FIG. 29. Freely growing bacilli 7 days after inoculation. A polymorphonuclear leukocyte has applied itself to right hand edge of the colony. Blue light. $\times 1500$.
- FIG. 30. Colony of freely growing bacilli being invaded and broken up by polymorphonuclear leukocytes. Omental spread made 7 days after inoculation. Blue light. $\times 1500$.
- FIG. 31. Same group of bacilli as shown in Fig. 30. Photomicrograph taken with white light to bring out polymorphonuclears. White light. $\times 1500$.



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Woodruff

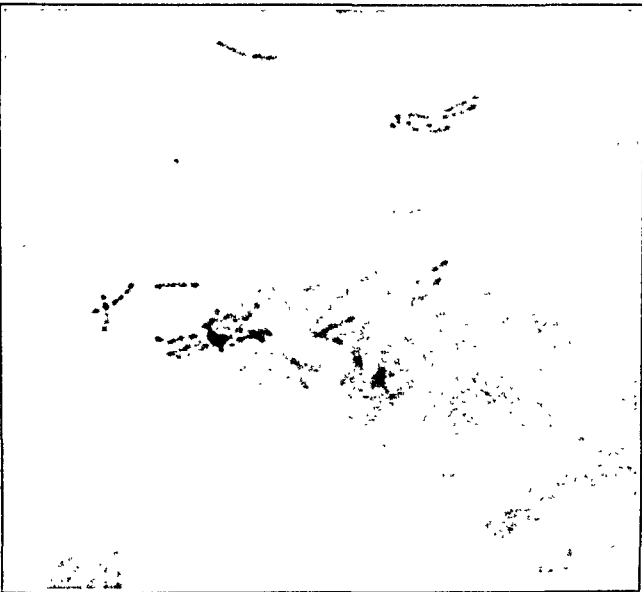


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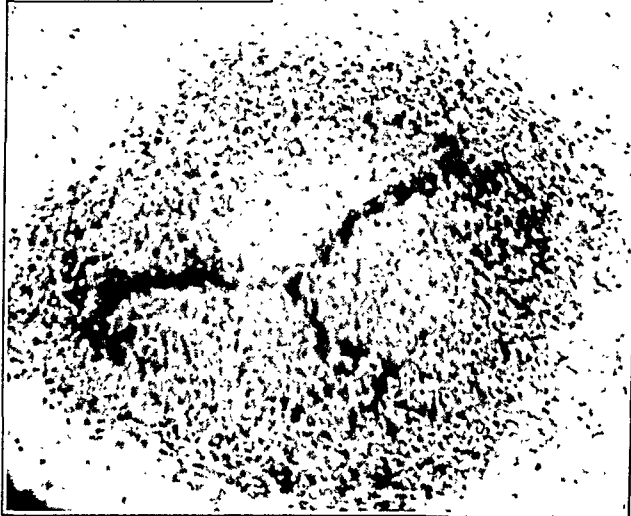
Free Growth Period of Tubercle Bacilli

PLATE 168

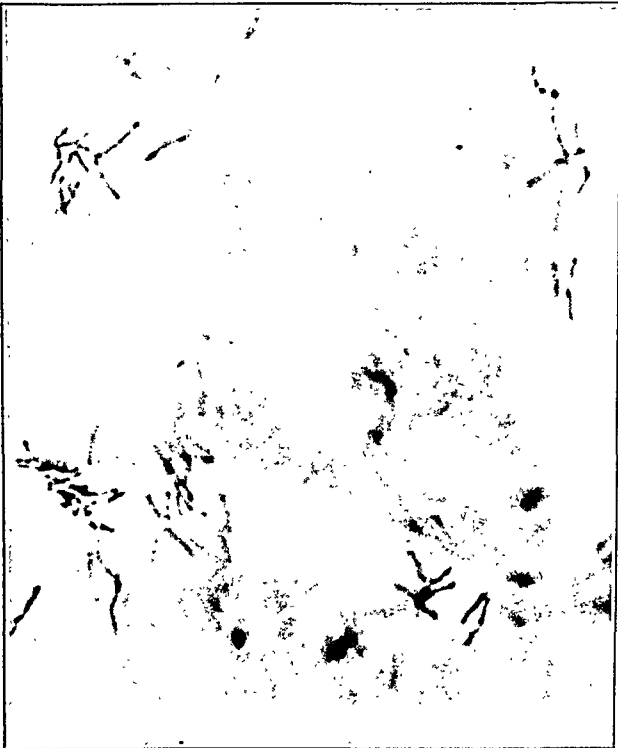
- FIG. 32. Showing marked beading of the freely growing bacilli, 7 days after inoculation. Blue light. $\times 1500$.
- FIG. 33. Two "vintages" of tubercles commonly found in omentum 9 days after inoculation. White light. $\times 165$.
- FIG. 34. Higher magnification of small tubercle shown in upper corner of Fig. 33. Blue light. $\times 1500$.
- FIG. 35. Tubercle bacilli of bizarre shape. From omental spread made 11 days after inoculation. Blue light. $\times 1500$.
- FIG. 36. Bacilli in omental spread made 37 days after inoculation. Blue light. $\times 1800$.



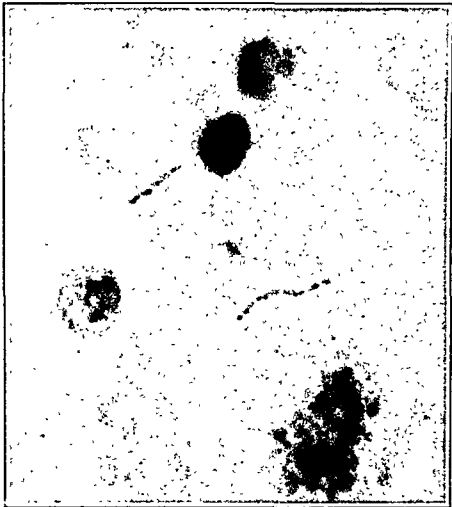
32



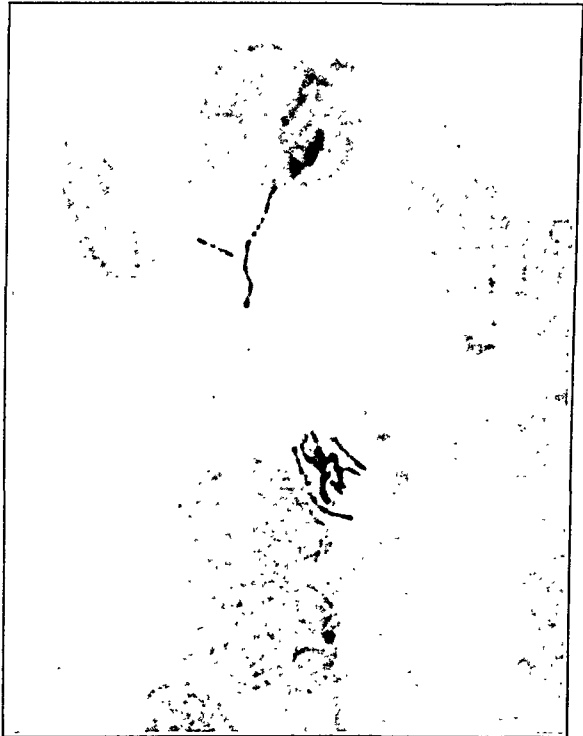
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SYPHILITIC ANEURYSM OF LEFT CORONARY ARTERY WITH CONCURRENT ANEURYSM OF A SINUS OF VALSALVA, AND AN ADDITIONAL CASE OF VALSALVA ANEURYSM ALONE *

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CORONARY ARTERY ANEURYSMS

The rarity of such aneurysms, exclusive of the false or dissecting forms and those associated with periarteritis nodosa, is stressed by Karsner¹ and further attested by the few reports in the literature. Packard and Wechsler,² 1929, in their classical survey, could find only 30 examples, the first of which was recorded by Bougon³ in 1812. Packard and Wechsler have thoroughly reviewed all reports of this condition, checked duplications and culled out questionable examples, so that we need only to cover the literature since 1929 and summarize briefly the salient facts elicited by these authors. They placed coronary aneurysms in two etiological groups: (1) mycotic-embolic, associated with bacterial endocarditis involving the aortic valve; and (2) arteriosclerotic, related to coronary sclerosis and long continued hypertension. The average age of the first group was 27 years, as compared with 57 years for the latter. There is no mention of syphilitic coronary arteritis as a cause of aneurysm of these vessels and mesaortitis was present in only 3 of the 30 collected cases. Rarely, indirect trauma due to strain may have been an etiological factor, but direct injury had an insignificant rôle, apparently on account of the protected anatomical site of the coronary vessels. In nearly all cases the aneurysms were single and located within the first inch of the left coronary artery, which was involved about three times as frequently as the right. In 4 hearts both coronaries were attacked. About 50 per cent of the aneurysms ruptured. There were no pathognomonic symptoms or signs that permitted an ante mortem diagnosis of the condition.

Cox and Christie,⁴ 1930, described a fusiform aneurysm 2.5 by 4.5 cm. involving the right coronary artery. This was associated

* Received for publication May 12, 1934.

with cardiac hypertrophy (500 gm.), old and organized thrombosis of the anterior interventricular branch of the left coronary artery, myocardial fibrosis and aneurysm of the abdominal aorta and of the right common iliac artery. Microscopic study of the aorta revealed only marked arteriosclerosis. Apparently no histological study was made of the coronary aneurysm. The lesion occurred in a white male 65 years old with a long history of vascular hypertension and of a paralytic "stroke."

Vogelsang,⁶ 1930, reported an aneurysm 6 by 5.5 by 4.5 cm. involving the anterior interventricular branch of the left coronary artery and situated in the anterior wall of the left ventricle. There were also gummatous myocarditis, syphilitic aortitis and gummatous pneumonitis. Distal to the aneurysm the artery was thrombosed. He included no microscopic description of the aneurysm, but expressed the opinion that in view of the gross and microscopic lesions elsewhere the aneurysm was probably also of syphilitic origin, and that the trauma of a 9 foot fall which the man, a 38 year old seafarer, sustained 4 months prior to his sudden death, might have been an etiological factor.

Halpert,⁶ 1930, described an arteriovenous communication between the right coronary artery and the coronary sinus, with aneurysmal dilatation of both, in a man 54 years of age who showed no cardiac disturbances during life and died of gastric carcinoma. From gross and microscopic investigation he concluded that the lesion probably was congenital.

Thus, to date (May 1934), 33 acceptable cases of coronary aneurysm have been recorded, and to this total we desire to add another, the only one of its kind among 5896 autopsies performed by the pathology department of the University of Oregon Medical School.

ANEURYSM OF THE AORTIC SINUSES OF VALSALVA

This is another uncommon site of aneurysm formation, but is less rare than in the coronary arteries. The comparative rarity of such lesions, together with the complications peculiar to their anatomical location, warrant the recording of further instances. In our autopsy material 2 cases have been encountered, one complicated by aneurysm of the left coronary artery, the other occurring in conjunction with syphilitic aortitis.

Valsalva aneurysms of congenital,⁷ syphilitic,^{8, 9, 10} arteriosclerotic,⁹ mycotic,^{9, 11} and indeterminate¹² etiology have been described as have dissecting or false aneurysms. Often these rupture, usually into the pericardial sac, the chambers of the heart or into the great vessels, but may burst externally after eroding the chest wall, as in the case of Sheldon,¹³ in which the pericardial sac had been obliterated by fibrous adhesions, presumably incident to rheumatic disease. Without rupturing, these aneurysms may burrow into the myocardium of the atria, ventricles or interventricular septum and in this way produce stenosis or insufficiency of one or more valves. Another complication, present in one of our cases, is heart block from encroachment upon the atrioventricular bundle, while still another, exemplified by our Case 1, is extension of the aortic disease to an adjacent coronary artery with the formation of an aneurysm in this vessel as well.

Most frequently the anterior sinus is involved, due, according to von Krzywicki,⁹ to its unsupported position superior and somewhat anterior to the membranous interventricular septum. Gray¹⁴ considers that the regurgitation of blood, directed chiefly against the anterior aortic wall, is a factor also.

The actual incidence of Valsalva sinus aneurysms is difficult to ascertain since most authors who have made statistical studies have not only failed to distinguish between true and dissecting aneurysms, but in addition have not made clear as to whether or not their figures are based only upon adequately described and complete autopsies. In many compilations the location of the aortic aneurysms is given only within wide anatomical limits. It is probable that some Valsalva aneurysms have been included with those of the ascending aorta and it is possible that others are buried in reports with misleading titles.

In order to obtain figures of incidence of various aortic aneurysms in a series of autopsies we have reviewed all of our records and have disregarded 605 protocols because the examinations were incomplete, or the description of the aorta was unsatisfactory. Among these were 13 cases in which one or more sacculations were present. Among the remaining 5896 autopsies 143 individuals had 214 true aneurysms of the aorta distributed anatomically as follows:

	No.	Per cent
Sinuses of Valsalva	2	0.93
Ascending aorta	102	47.66
Ascending aorta and transverse arch	11	5.14
Transverse arch	35	16.35
Arch and thoracic	1	0.47
Ascending, arch and thoracic	3	1.40
Thoracic	43	20.10
Abdominal	17	7.94

In 20 persons there were 26 dissecting aneurysms which are not included in the tabulation. Among those listed were 6 cases of concomitant true and dissecting aneurysm, but the latter have been omitted from the compilation. In the tabulated group the probable etiology was: syphilitic 87.8 per cent, arteriosclerotic 10.3 per cent, mycotic-embolic 1.4 per cent and rheumatic 0.47 per cent. These figures agree fairly well with those of Brindley and Schwab,¹⁵ but in our series syphilis seems to be a somewhat more prominent etiological factor, possibly because only true aneurysms are included.

We have made no exhaustive survey of the literature dealing with aneurysms of the sinuses of Valsalva but wish to call attention to the statistics of Smith¹⁰ and of Lucké and Rea¹⁶ who found 10 cases among 287 aortic aneurysms in 12,000 autopsies collected from various sources.

CASE REPORTS

CASE 1. *Syphilitic Aneurysm of the Left Coronary Artery with Concurrent Aneurysm of Sinus of Valsalva:* The clinical history and autopsy record were not available.

Postmortem Examination

The heart and 6 cm. of the ascending aorta had an aggregate weight of 595 gm. The intima of the ascending aorta was wrinkled, roughened, pearly gray to whitish and mottled by irregular yellowish areas. The wall was irregularly thickened, of cartilaginous consistence and in a state of saccular aneurysmal dilatation. The epicardium and endocardium were grossly unchanged. The left ventricle was greatly hypertrophied and dilated. The trabeculae carneae and papillary muscles were much enlarged, elongated and flattened. The mitral leaflets were unchanged. The aortic leaflets felt gristly and had thickened, rolled, rounded edges. The aortic ring had a circumference of 8.5 cm. The left atrial wall was 3 to 4

mm. thick across the pectinate muscles, but between them was almost transparent. There were two right coronary ostia, situated 2 mm. apart. The smaller was less than 1 mm. in diameter and led to a vessel coursing over the conus arteriosus. The orifice of the main artery was slit-like and measured 1 by 3 mm. Both ostia opened 1.1 cm. superior to the upper border of the anterior aortic leaflet. Serial cross-sections of the artery revealed some eccentric thickening of its wall by atherosclerotic plaques which did not close the lumen.

In the left posterior aortic sinus was an aneurysm having a crescentic opening measuring 0.7 by 1.8 cm. Within the anterior wall of the left ventricle this aneurysm expanded, attained dimensions of 3.1 by 3.4 by 2.4 cm. and became filled by a laminated thrombus. Anteriorly and to the right the sac bulged into the right ventricle directly below the posterior leaflet of the pulmonic valve, elevating it somewhat, and producing a triangular stenosis of the valve. The base of the triangle was formed by the bulging aneurysm wall and the opening of the valve was reduced to about half its normal size. The lining of the aneurysm resembled that of the aorta. The inferior wall of the sac was formed by the left ventricular myocardium and did not encroach upon the membranous part of the interventricular septum, but lay somewhat anterior to it. The sac also bulged into the left atrium beneath the anterior leaflet of the mitral valve, elevating it to some extent and producing a slight degree of stenosis. The right ventricle was markedly dilated and its wall was considerably hypertrophied.

The left coronary artery and the Valsalva aneurysm were examined by gross serial cuts, as shown in Figure 3. The left coronary ostium (Fig. 3A) lay 0.7 cm. above the tip of the left posterior aortic leaflet, was oval, measured 3 by 5 mm. and had its long diameter in the superior-inferior direction. It was surrounded by pearly white, wrinkled and greatly thickened aortic intima. Within 5 mm. of its origin (Fig. 3B) the main trunk enlarged to an outside diameter of 2.1 by 0.9 cm. Adherent to the rigid intima was a film of dry, blackish blood clot and even at this point the inferior half of the lumen was occluded by a laminated brownish gray thrombus. The vessel wall was from 1 to 3 mm. thick and fused with the aortic wall. Six mm. distal to the point just described (Fig. 3C) a marked change occurred. The coronary artery was now 2.3 cm. in one diameter and 2 to 4 mm. in the other. The wall had a thickness of 1 to 2 mm. and

over the superior part the lining was coated with blackish blood. Inferiorly the vessel came to a sharp point and here the thrombus observed in the previous block plugged the lumen. In the middle of this segment was the ostium of the circumflex branch of the left coronary artery, also occluded by the thrombus. It must be understood, then, that the dimensions given above represent not only the main coronary artery but also the beginning of its circumflex branch, and because this was cut tangentially the lower part of its lumen appeared pointed. On the right side of the coronary artery lay the Valsalva aneurysm containing a laminated thrombus, most of which dropped out in the process of sectioning. The coronary artery and the Valsalva aneurysm were separated by a hard and fibrous wall only 2 mm. thick. Four mm. distally (Fig. 3D), measuring along the coronary artery, was the beginning of the anterior interventricular branch. The main left vessel was still rather larger than normal, with an external diameter of 1.1 by 0.7 cm., but had decreased appreciably from its size in the preceding block. Its lumen had an undulating outline and the intima was covered by a thin, blackish film. The common wall dividing the artery and the Valsalva aneurysm appeared wholly fibrous and was 2 to 3 mm. thick. At this point the Valsalva sac measured 2.3 cm. in its transverse diameter, 3.4 cm. in the supero-inferior direction and had a wavy and whitish border. In the myocardium of the left ventricle forming the lower border of the aneurysm were many engorged capillaries within a whitish scar. In this block the circumflex artery had fully emerged as an independent vessel and exhibited nothing abnormal. The thrombus occluding the beginning of this vessel did not continue further along its course. The myocardium under the coronary aneurysm was only 6 mm. thick. The anterior interventricular branch, 5 mm. distal to the point previously described (Fig. 3E), was shaped like a bowling pin, with maximum external diameters of 1.2 and 0.6 cm. Its lumen was entirely closed by a dry, blackish clot. The same common wall, still 2 mm. in thickness, separated the vessel and the Valsalva aneurysm, which now measured 3.5 by 2.5 cm. The latter had become more superficial and was underlaid by 1.1 cm. of myocardium. The myocardial scar mentioned above continued into this block. The outside dimensions of the coronary artery were now 7 and 9 mm. In the next block (Fig. 3F), having a thickness of 5 mm., the Valsalva aneurysm had practically left the myocardium

and had come to lie chiefly in the subepicardial fat. Its dimensions were decreasing, being 3 by 2 cm. The wall of the sac and the thrombus contained within it were identical with previously mentioned blocks. The myocardial scar, however, had practically disappeared. The coronary artery and the Valsalva aneurysm continued to share a common wall and the thrombus occluding the lumen of the artery in more proximal blocks was still present. The external diameters of the vessel were the same as in the preceding block although the contour was different. Within the next 5 mm. (Fig. 3G) the Valsalva aneurysm had fully emerged from the myocardium, lay wholly in the subepicardial fat and decreased in size to 2.3 by 1.8 cm. The walls of the anterior interventricular artery and the aortic sinus aneurysm were still in apposition but no longer fused into one. The thrombus mentioned previously continued and the coronary artery measured 5 by 7 mm. Three mm. distally (Fig. 3H), the Valsalva aneurysm terminated blindly in the subepicardial fat, 4 mm. beneath the epicardium, and had not ruptured. The relations of the two aneurysms are shown clearly in the wax reconstruction (Fig. 2).

Microscopic Examination

Microscopically the clots filling the Valsalva sacculation and the aneurysm of the left coronary artery prove to be typical laminated thrombi exhibiting some softening but no organization.

The wall of the Valsalva aneurysm consists almost entirely of hyalinized fibrous connective tissue in which the Van Gieson and Verhoeff stains display persisting remnants of both smooth muscle cells and elastic tissue. Numerous partially or completely obliterated vasa vasorum are collared by abundant plasma cells and lymphocytes. The medial and intimal divisions are not clearly distinguishable on account of the great distortion and fibrosis, while in the inner part of the wall there are extensive atherosclerotic changes and calcification. The common wall separating the Valsalva and coronary artery aneurysms is made up of hyalinized fibrous tissue and granulation tissue containing fragments of elastic and smooth muscle tissue. Here also are small vessels showing obliterative changes and perivascular cuffs of lymphocytes and plasma cells. It appears obvious that this common wall represents a fusion of the walls of the aneurysmal aortic sinus and the left coronary artery.

The left coronary artery wall exhibits changes identical with those just described for the Valsalva aneurysm and these are depicted in Figure 4. The myocardium subjacent to the aortic sinus aneurysm gives histological evidence of pressure atrophy, hyaline degeneration, interstitial fibrosis and contains numerous small and engorged blood vessels. Other sections of the myocardium reveal a distinct hypertrophy of the muscle cells with deposits of lipochrome pigment at either end of the nuclei, slight patchy interstitial fibrous connective tissue increase and multiple small areas of anemic necrosis in blocks coming from the anterior wall of the left ventricle. The intima of the ascending aorta is the seat of typical atherosclerosis with calcification, while the media displays extensive destruction of smooth muscle and elastic tissue. The vasa vasorum of both media and adventitia are greatly narrowed or obliterated by endothelial proliferation and intimal fibrosis and are collared by plasma cells and lymphocytes. Some of these accumulations show early necrosis and are regarded as actual miliary gummas.

CASE 2. *Syphilitic Aneurysm of Aortic Valsalva Sinus:* The patient, a white male, 60 years old, spoke and understood so little English that an adequate history was unobtainable. He had been working "in the woods," presumably at logging, until about the first of November, 1932, when he became short of breath, progressing to orthopnea within 2 months. About the first of January, 1933, there developed a continuous, dull, distressing pain in the epigastrium and right upper abdomen. Swelling of the ankles appeared about a month later. He was weak, "nervous," could not sleep, and had been confined to bed most of the time since the appearance of the edema. Occasionally he had noted substernal distress and tinnitus. He was admitted, walking, to Multnomah County Hospital on Feb. 21, 1933.

On admission the temperature was 95 F, pulse 88, respiration 24 and the blood pressure 160/35. Physical examination revealed slight pallor and cyanosis of the finger nails, but no demonstrable capillary pulse. The retinal blood vessels pulsated and the optic discs appeared hazy. The neck vessels pulsated markedly with systole. The chest was slightly emphysematous. The cardiac impulse was diffuse and heaving. There was slight impairment to percussion between the right scapula and the spine, with bronchovesicular breathing and a to-and-fro murmur. Elsewhere the lung fields seemed normal. To percussion the heart was of the "aortic" configuration, the arch 6 cm. wide, and the left and right borders respectively 14 and 6 cm. from the midsternal line. A loud to-and-fro murmur was audible over all valve areas, loudest at the aortic and mitral areas and transmitted to the axilla and through to the back. No distinct valve tones could be heard. The peripheral vessels were thick-walled and pulsated forcibly. The abdomen was distended. The liver border was 6 cm. below the costal margin and was felt also in the epigastrium. The legs were very edematous.

The urine was negative. Except for a sedimentation rate of 13 mm. in 15 minutes and 37 mm. at the end of 45 minutes (modified Westergren method), and 4 plus Kolmer and Kahn reactions, examination of the blood yielded results within normal limits.

An electrocardiogram taken on the day of admission showed the auricular and ventricular rates to be each 71. T_1 was inverted, R_1 slurred, Q-S prolonged and the P-R interval prolonged to 0.27 second. T_2 was inverted, R_2 slurred, and P-R prolonged. R_3 was slurred and notched, P_3 and T_3 were questionably diphaseic and R_3 of low amplitude. The interpretation was: "Delayed A-V conduction, coronary type T-waves, myocardial damage."

Under a regimen of bed rest, digitalis, sedatives, bismuth and iodides, the patient improved and 3 days later the peripheral edema was gone. On February 27th a roentgenogram of the chest showed increased hilum shadows, pleural thickening on the right, with obliteration of the costophrenic angle, and a greatly enlarged cardiac shadow with a blunt apex and a somewhat widened and sclerotic arch.

By March 2nd there was no dyspnea or cyanosis. The man's condition remained unchanged until April 15th, when he had diarrhea and abdominal distress. Six days later he suddenly became cyanotic and died.

The final clinical diagnoses were: (1) Aortic regurgitation on the basis of syphilitic destruction of the aortic ring; syphilitic aortitis; hypertrophy and dilatation of the right and left heart with cardiac failure, functional capacity II-B, and peripheral edema. (2) Mild chronic hypertrophic emphysema.

Postmortem Examination

No. 163-4-33. Examination of the abdomen revealed slight ascites, a non-specific diphtheritic, hemorrhagic and ulcerative enterocolitis and proctitis, and chronic passive hyperemia of the liver with acute periportal hepatitis, and chronic passive congestion of the spleen and kidneys.

The left pleural cavity contained about 3 liters of clear fluid, which compressed the lung inferiorly and posteriorly. The right lung was bound to the chest wall by dense adhesions at its apex, and laterally and inferiorly over its middle and lower lobes. Some of these adhesions, which were fibrous in nature, obliterated the right costophrenic sinus and encapsulated some yellowish white, cheesy and hyaline material. The space occupied by the heart was greatly increased, having a maximum transverse diameter of 19 cm. All cardiac chambers were much dilated and more than half of the anterior presenting surface was formed by the right ventricle and atrium. The papillary muscles and trabeculae carneae of both ventricles were appreciably elongated, thickened and also flattened.

With the heart opened there could be seen immediately inferior to the pulmonic valve a rounded, bulging area forming a sort of

shelf on the septal wall of the right ventricle and producing some stenosis immediately proximal to the valve. Over the inferior half of this projection the endocardium was pearly white and the superior border of the whitened area was quite sharp, due to the fact that at this point the interventricular septum had been reduced to a hyaline state and formed one wall, here only 2 mm. thick, for the Valsalva sinus aneurysm to be described in more detail presently. Several of the chordae tendineae of the anterior cusp of the tricuspid valve were attached to the aneurysm wall which lay immediately anterior and to the left of this valve. The aortic valve measured 9 cm. in circumference and its leaflets were somewhat rigid, with thickened and rolled margins. The commissures between the leaflets were widened, the distance between the right and left posterior leaflets being 1 mm., while the commissures separating the anterior and left posterior leaflets and the anterior and right posterior leaflets were each 3 mm. At a point 4 cm. superior to the aortic ring the aorta had a circumference of 12.5 cm. The ostium of the right coronary artery was slit-like, measured 1 by 2 mm. and was situated 6 mm. superior to the tip of the anterior aortic leaflet on the superior aspect of the shelf-like margin of a semilunar aneurysm 2 by 1.3 cm. in size, which occupied the right posterior sinus of Valsalva and extended into the interventricular septum for a distance of 2.8 cm. (Fig. 5). In its development the aneurysm left behind a narrow and firm ridge separating it from the attachment of the right posterior aortic leaflet. The lining of the sac was rough, grayish white, mottled by yellowish atheromatous plaques, of cartilaginous firmness and displayed over a part of its blind end a thin, grayish red thrombus. The wall forming the blind end lay in a concavity hollowed out of the muscular interventricular septum. The pars membranacea septi appeared to have been displaced to the left to form most of the left wall of the aneurysm, which, however, did not stop here but continued downward, cupping out for itself a bed in the muscular interventricular septum. Along the right border of the shelf bearing the right coronary orifice was a deep and narrow vertical groove communicating directly with the Valsalva aneurysm. The ostium of the left coronary artery measured 2 by 3 mm. and opened 2 cm. superior to the upper boundary of the left posterior aortic leaflet. Serial cross-sectioning of the coronary arteries revealed them to be macroscopically unchanged. The wall of the en-

tire aorta was irregularly thickened and distorted and the vessel was quite tortuous. The intima, from the root to the bifurcation, was whitened, thickened and wrinkled longitudinally. Toward the bifurcation was an increasing amount of atheromatous change with ulceration and calcification. The vessel cut with leathery resistance and was unduly adherent to the structures about it. In the anterior wall of the abdominal aorta was a small saccular outpouching. The heart and entire aorta weighed 940 gm. The myocardium of both ventricles was distinctly hypertrophied and pinkish, with a slight yellowish mottling. The anterior wall of the left ventricle seemed to be elongated. Fibrotic patches were noted at the tips of the various papillary muscles. The mitral valve ring was 11.5 cm. in circumference and its leaflets, particularly the anterior one, were flecked by atheromatous patches.

In the lower lobe of either lung were several rubbery, yellowish to grayish, sharply circumscribed, grouped nodules with polycyclic outlines. The bronchial and pulmonary arterial walls were thickened and some of the latter displayed atheromatous plaques.

Microscopic Examination

Microsections of the septal portion of the Valsalva aneurysm reveal a partially organized and partly softened thrombus at its base. The wall of the sac is formed by a thick layer of hyaline material showing atheromatous changes and hemosiderin deposits. Blending with this is a layer of vascular granulation and fibrous scar tissue containing numerous partially or completely obliterated arteriolar channels surrounded by broad collars of lymphocytes and plasma cells. By means of the Verhoeff stain fragments of degenerating elastic tissue are identified, proving that this portion of the sac represents remnants of aortic media. Separating this layer and the septal myocardium is a thin zone of connective tissue, probably representing aortic adventitia, containing a number of tiny vascular channels, apparently veins. Farther out are degenerated cardiac muscle cells, isolated or split up into small groups and compressed by dense fibrous tissue. On account of the formalin fixation and the time elapsing between death and autopsy, special stains to demonstrate the cells of the atrioventricular bundle were not employed.

Sections of the aorta, taken at various levels from the root to the bifurcation, all exhibit essentially the same changes, namely, irregular thickening, fibrosis, puckering and distortion of all layers, with extensive fragmentation and destruction of medial smooth muscle and elastic tissue with partial replacement of these by fibrous connective tissue of varying age. The aortic vasa vasorum display all degrees of obliterative endarteritis and are surrounded by varyingly dense collars of lymphocytes, plasma cells, Russel-Plimmer bodies and occasional polymorphonuclear eosinophiles. Certain of the cellular accumulations show slight necrosis. The adventitia is the seat of similar changes.

Sections of myocardium from either ventricle exhibit marked hypertrophy and deposition of pigment at the nuclear poles with distinct cross-striations in some cells and an absence of these in others. A few of the cells appear to be atrophic. The interstitial tissue seems to be slightly edematous, a little increased in places and contains occasional lymphocytes and plasma cells but no cellular aggregations resembling gummas. The smaller branches of the coronary arteries are unchanged. Frozen sections stained with scharlach R fail to show any fat in the myocardium.

Microscopic examination of the lower lobes of the lungs reveals compression atelectasis, chronic passive hyperemia, edema, slight emphysema, and numerous pneumoliths. Here and there, in relation to bronchi or larger blood vessels are nodules composed of plasma cells and lymphocytes, surrounded by vascular granulation tissue containing many of these cells. Large areas of incomplete caseation necrosis in which the architecture of lung tissue and blood vessels can still be recognized are present. The necrotic tissue stains pinkish with eosin and there is no evidence of calcification or persistence of nuclear fragments, nor are any giant cells or epithelioid cells seen. No structures resembling daughter tubercles are in evidence. At the edges of such lesions the arterioles have undergone extensive obliterative inflammation. The larger branches of the pulmonary arteries are atheromatous and proliferative intimal changes have reduced their lumens to some extent. In places their walls contain a moderate number of lymphocytes, plasma cells, and rarely eosinophilic and neutrophilic polymorphonuclears. The right costophrenic pleural thickening consists of hyalinized fibrous

tissue and granulation tissue containing patchy accumulations of lymphocytes and plasma cells, forming a capsule about amorphous and hyaline material in which there is much cholesterin.

DISCUSSION

In addition to the previously recognized causes for the development of aneurysm of the coronary arteries, such as mycotic-embolic infection and arteriosclerosis, we offer another, namely, syphilitic arteritis. In so doing we are cognizant of the truth of the generally accepted belief that the coronary arteries are only rarely affected by syphilis distal to their intra-aortic segments. However, in Case 1 there exists a most unique pathological condition which modifies the usual circumstances to such an extent that we have no hesitancy in terming the coronary lesion syphilitic. Undoubtedly the involvement of the left coronary artery was dependent first upon the localization of an active syphilitic aortitis in the left posterior sinus of Valsalva, and secondly the direction of burrowing of the enlarging sac which finally brought it into intimate contact with the main left coronary artery. There must then have been a spread of the *Spirocheta pallida* from the wall of the Valsalva sac to the wall of the coronary artery, with resultant destruction, fusion of the walls of the two juxtaposed structures and, finally, the formation of a true aneurysm in the weakened coronary artery.

As evidence of the syphilitic nature of the lesion we submit the microscopic observation of obliterative endarteritis of the vasa vasorum, perivascular collars of plasma cells and lymphocytes, microscopic sized gummas, destruction and scarring of the media and adventitial fibrosis, all of which are recognized as characteristic of syphilis by Warthin¹⁷ and Moritz.¹⁸ A careful study of the aorta, coronary arteries and myocardium failed to disclose anything that could be interpreted as rheumatic disease, a possibility which, in view of the newer histopathology of rheumatism, as described by Klinge and Vaubel,¹⁹ and others, must be kept constantly in mind in the study of vascular lesions. It should be mentioned also that the vascular changes in the coronary artery of Case 1 were quite different from the commonly observed adventitial cellular infiltration accompanying coronary arteriosclerosis.

Another feature of this case makes it doubly interesting, for in addition to aneurysm of the coronary artery there was recent thrombotic occlusion of the sacculation and the lumen of the vessel adjacent to it. Closure of a main coronary artery near the heart would lead to serious consequences, even in a healthy organ, and in this instance would be even more embarrassing on account of the previously existent and plainly evident aortic insufficiency and dilatation of the left ventricle. Although the record and identifying number of the specimen are missing there is every reason for believing that coronary thrombosis was the terminal event of this person's life. Death evidently supervened shortly after the thrombus formed, for only the earliest indications of infarction of the myocardium were present.

While Vogelsang's ⁵ example of coronary aneurysm may well have been due to syphilis, one cannot be certain that such was the case because the vessel was not studied histologically. In our case, although spirochete stains were not done, it is felt that the evidence of syphilis is indisputable. With the possible exception of Vogelsang's case the present example appears to be the first of its kind thus far recorded.

In Case 2 it is possible that the incipient heart block, not evident clinically but suggested by the prolonged atrioventricular conduction time and the inverted T-waves of the electrocardiogram, may have been caused by digitalis, but we feel that the Valsalva aneurysm, on account of its size and anatomical relation to the atrioventricular bundle, together with degenerative and fibrotic changes in the adjacent myocardium, afford a more plausible explanation for these phenomena. Another factor contributing to cardiac failure was aortic insufficiency, which not only threw additional strain on the left ventricle but also failed to allow sufficient blood to enter the stenosed coronary ostia to nourish the myocardium properly. The right cardiac hypertrophy probably was due to increased pressure in the lesser circulation bed, due both to the stenosis of the pulmonary valve region by the Valsalva sinus aneurysm and also to pulmonary atherosclerosis which, in turn, probably followed increased circulation pressure from left heart failure on the basis of aortic insufficiency and stenosis of the mitral area from the Valsalva aneurysm. The presence of plasma cells and lymphocytes in the walls of the pulmonary arteries suggests that the sclerosis of these vessels may, in part at least, have been the result of syphilis.

The lesions in the bases of the lungs exhibited the characteristics of gummas and bore scarcely any resemblance to tubercles. Although the spirochete stains failed to demonstrate the organism it is our opinion, from the histological structure of the lesions and the known syphilitic nature of the aortic disease, that the pulmonary foci are very probably luetic as well.

The aortic involvement was much more extensive than is usual in syphilis and it is interesting to note that a second saccular aneurysm had developed in the abdominal division of the vessel.

SUMMARY

Two cases of syphilitic aneurysm of the aortic sinuses of Valsalva with unusual complications are described. Such sacculations are distinctly uncommon, forming only 0.93 per cent of the aortic aneurysms in our series of 5896 autopsies.

In 1 case the aneurysm burrowed through the ventricular myocardium until it reached the left coronary artery, where a secondary syphilitic arteritis was established, leading first to aneurysm of the coronary artery and finally to acute thrombotic occlusion. This is the 34th case of coronary aneurysm to be recorded and apparently the first to have a syphilitic etiology.

The uncommon manifestation of Valsalva aneurysm in the second case was incipient heart block, dependent upon the proximity of the sac to the atrioventricular bundle.

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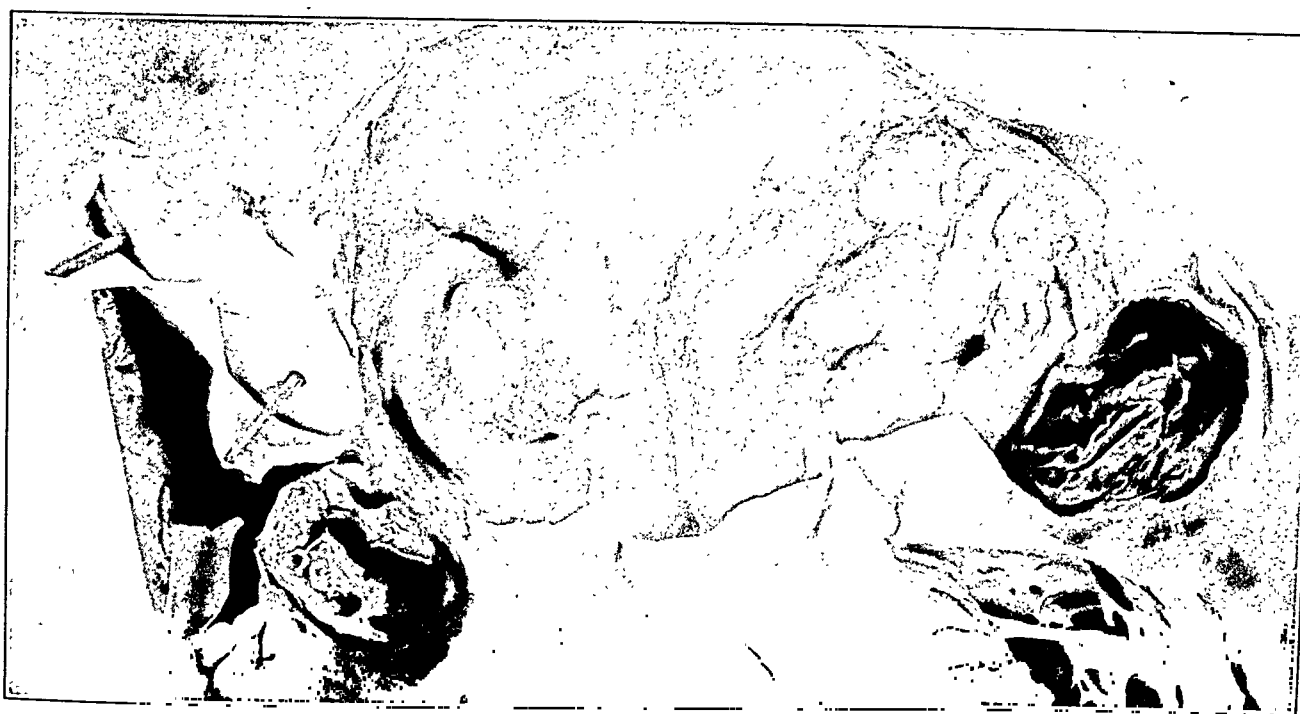
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DESCRIPTION OF PLATES

PLATE 169

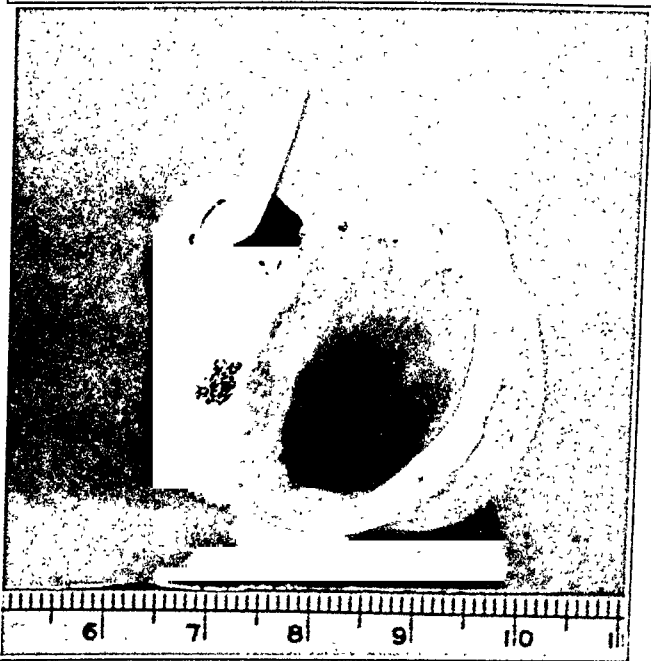
- FIG. 1. Photograph of a portion of the left ventricle and aorta of Case 1. To the left are two right coronary ostia and a portion of the thrombosed Valsalva aneurysm. Above and to the extreme left are the right and left anterior pulmonic leaflets held out by props. To the right is the left coronary ostium and the Valsalva aneurysm showing its direct connection with the aorta and its roof formed by the beginning of the pulmonary artery. The groove to the right of the sac lies between the lateral wall of the pulmonary artery and the left anterior leaflet of the pulmonic valve.
- FIG. 2A. Wax reconstruction of Valsalva (white) and left coronary (black) aneurysms viewed anteriorly. Natural size. A white marker has been placed in the ostium of the artery. To the right is the beginning of the circumflex branch. The flatly oval shape of the coronary aneurysm is well shown in this view.
- FIG. 2B. Wax model viewed from above showing the cavity of the Valsalva aneurysm. The white area toward the base of the coronary artery is a highlight.



I



2A



2B

PLATE 170

FIG. 3. Case 1. Drawing of the two aneurysms. Actual size. The distal face of each segment excepting Block 1 is depicted. Thus if 11 were superimposed upon 6 and so on, the aneurysms would appear as they were before sectioning.

FIG. 3A. Ostium of left coronary artery.

FIG. 3B. Block 5 mm. distal to ostium of left coronary artery.

FIG. 3C. " 11 mm. " " " " " " " "

FIG. 3D. " 15 mm. " " " " " " " "

FIG. 3E. " 20 mm. " " " " " " " "

FIG. 3F. " 25 mm. " " " " " " " "

FIG. 3G. " 30 mm. " " " " " " " "

FIG. 3H. " 33 mm. " " " " " " " "

a, ostium of left coronary artery; *b*, pulmonary artery; *c*, left atrium; *d*, mitral valve cusp; *e*, Valsalva aneurysm; *f*, left main coronary artery; *f'*, anterior interventricular branch of left coronary artery; *f''*, circumflex branch of left coronary artery; *g*, left ventricular myocardium; *h*, myocardial scars; *x* *x'*, and *x' y*, indicate boundaries of areas from which blocks for microscopic study were taken.

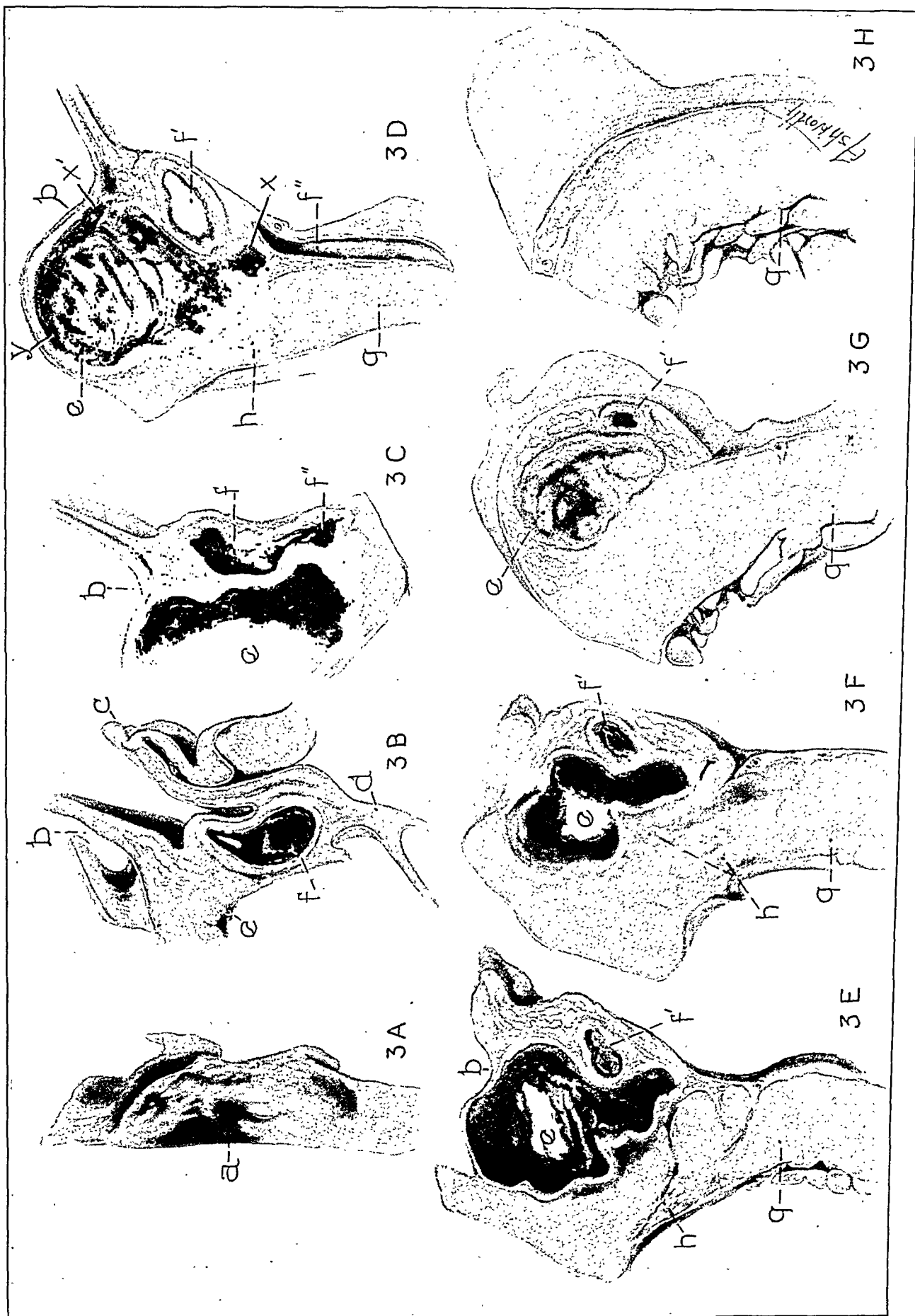
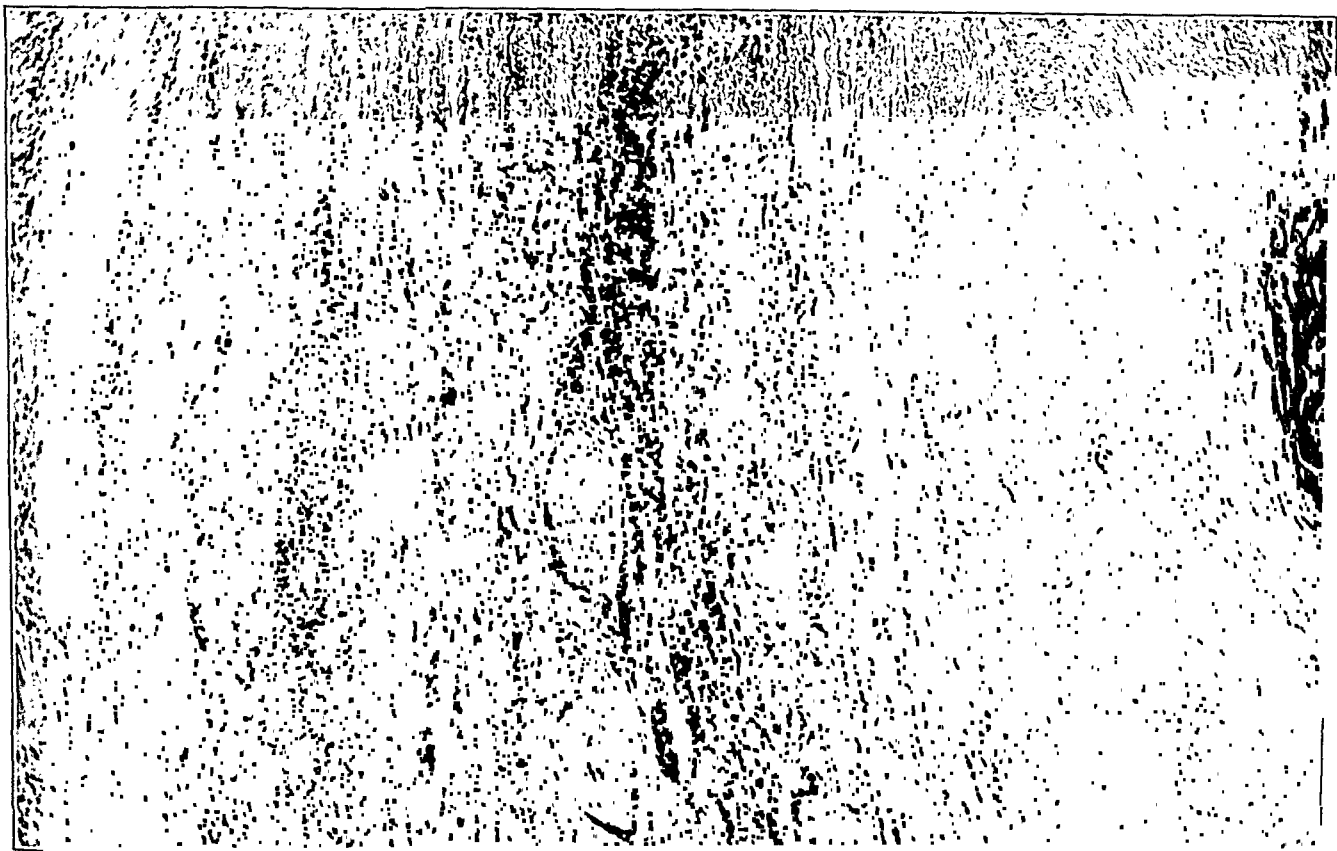


PLATE 171

FIG. 4. Case 1. Low power photomicrograph showing the intima, media and a small portion of the adventitia from the left coronary artery aneurysm near the point where it fuses with that of the sinus of Valsalva. Along the right margin is a small part of the occluding thrombus. The intima is thickened and contains a small capillary. Much of the media has been destroyed and is heavily infiltrated with lymphocytes and plasma cells. A nutrient vessel with marked narrowing of its lumen is shown and above it is a tangentially cut nerve. Note also the cellular infiltration and fibrosis of the adventitia at the extreme left.

FIG. 5. Case 2. View of the Valsalva aneurysm after opening the left ventricle and aorta. A part of the wall of the aneurysm has been cut away and turned to the right in order to display the bed of the sac in the interventricular septum. Near the left border of the aorta is the orifice of the left coronary artery. Note the thickening and distortion of the intima in the ascending aorta.



4



5

ADAMANTINOMA OF THE UPPER JAW *

REPORT OF A CASE

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Prior to the publication of Malassez' ¹ work the grouping together of certain tumors of the jaws, as having a possible common origin in the gingivodental epithelium, may be said to date from the theory advanced by Verneuil (cited by Charvot ² and Cumston ³) that all growths of epithelial character in the jaws, especially multilocular cysts, adenomatous cysts and the periosteal cysts of Magitot, ⁴ arose in cell masses which he called "paradental débris." However, the fact that Malassez' theory was widely known in France before 1881 is evidenced by Cumston's reference to it. Certainly by that time Falkson, ⁵ in 1879, had advanced the theory of enamel organ origin and the hypotheses of Broca ⁶ and Magitot ⁴ had already been accepted by many authors. The first description of growths of this type is found in Scultet's *Armamentarium Chirurgicum*, 1655 (pages 222-228), and the term adamantinoma was first suggested by Goebal ⁷ in 1897, and reintroduced by Ferrero ⁸ in 1906, to denominate tumors composed of a fibrous stroma and epithelium arising from that of the gingivodental tract. In this way the term has been used for growths such as those reported by Jeannel, ⁹ Péan, ¹⁰ Brown ¹¹ and others, where only epithelium of the malpighian type was present and no trace of true adamantine cells could be found. In many instances, however, its application was limited to epithelial growths where no adult dental tissue could be detected. Coryllos, ¹² and many French authors, on the other hand, have used it to cover all epithelial growths originating in the gingivodental tract irrespective of their contents. It is doubtful whether there exists any basis in fact for separating "dental cysts," that is, cysts in association with an erupted tooth, and "dentigerous cysts" (in which a non-erupted tooth is included in a growth of this type) from the

* Received for publication June 21, 1934.

group of adamantinomas proper, the former as inflammatory proliferations of the epithelial débris of Malassez, and the latter as of follicular origin. Coryllos¹³ reports the occurrence of a growth of typical differentiated adamantine cells in a patient who had suffered no dental trouble prior to the age of 35 years, at which time a carious molar was extracted, followed by the development of the tumor. Both the temporary and permanent dentitions had been normal and complete. Origin by proliferation of the paradental débris of the affected tooth appears most probable in this case. Heath¹⁴ states that so-called dental cysts may develop into multilocular, or even solid adamantinomas, if not completely extirpated. In the Report on Odontomes¹⁵ is found the statement that, in the case of all such cysts, to avoid recurrence the entire epithelial lining must be destroyed. Kegel,¹⁶ and others, believe that in such instances of either a multilocular or solid adamantinoma it is the presence of the growing tumor of dental germ origin that is responsible for the dental crises, and consequently for the tooth extraction, and that it is not irritation of the paradental débris by inflammation and suppuration, and later damage to the débris during extraction, that is responsible for subsequent tumor development. They cite in support of this the fact that fluid frequently escapes at the time of extraction. The fifth case recorded by Charvot would appear to refute this theory, as the patient insisted that at the time of extraction of the offending tooth no fluid escaped, although 6 months later, when an obvious swelling had developed at that site, fluid containing cholesterol crystals was drained from what proved to be a cystic adamantinoma in the tooth socket.

Malassez and Coryllos affirm that an adamantine growth formed around an unerupted tooth the root of which is developed, originates not in the follicular epithelium but in the gubernacular portion of the paradental débris (probably the remains of the cord of the enamel organ), by an excessive proliferation and cystic degeneration, which normally would bring about the eruption of the tooth. In the case of the permanent teeth, with the exception of the first molar which erupts like the deciduous teeth, malformation of the *iter dentis* or bony canal by which the tooth erupts may be the irritating factor, as first suggested by Albarran¹⁷ (Fig. 2). Certainly in the Report on Odontomes it is stated that in some cases of dentigerous cyst "absence of the corresponding tooth has been assumed without reason,"

and that where a perfect tooth was included its root was as a rule embedded in the cyst wall. Coryllos points out that were such tumors of follicular origin the tooth might be expected to lie entirely within the cyst cavity. He further affirms that in the only true dentigerous tumor of follicular origin the crown only of the included tooth is formed, the tumor originating in the epithelium of Von Brunn's sheath, the sole portion of the enamel organ remaining after development of the tooth crown. Cases such as those reported by Remy¹⁸ and Duret,¹⁹ where the included wisdom tooth was covered by Nasmyth's membrane, so that all epithelial elements of the dental follicle were intact, may be quoted in support of this theory. Nor are such growths always of predominantly cystic character.

The classification of Coryllos, including as adamantinomas all jaw growths of dental origin, with a persistent epithelial factor, irrespective of the contents, would appear to have the merit of simplicity and logic. The only point to which exception may be taken is the inclusion of growths of purely malpighian type of epithelium exhibiting epithelial pearls and areas of cornification. Both the inner and outer layers of the enamel organ are formed by basal cells (Fig. 1), and one may therefore assume that the basal cells of the gingivodental tract may undergo one of two forms of evolution — stratification, as in the formation of gingival mucosa, or cylindrization and enamel secretion. No evidence has yet been offered to show that cylindrical ameloblasts may be transformed into malpighian cells. If the term adamantinoma is to be limited to growths exhibiting epithelium in the line of development toward enamel formation, tumors of the purely malpighian type, arising as they must in certain sections of the paradental débris, may better be termed "paradental acanthomas."

Most writers agree with the statement of Bloodgood,²⁰ that conservative operations tend greatly to increase the rate of growth and enhance the penetrative capacity of these neoplasms in the recurrences (stated by Kegel¹⁶ to follow in 76 per cent of cases, and reported by Simmons²¹ as following in every one of 10 cases in which an incomplete operation was carried out). However, it is of interest to note that Hautant,²² in reporting a case of adamantinoma of the upper jaw involving the middle meatus and the maxillary antrum, regrets that he did a maxillary resection, since he did not take into

consideration the relation of the growth to that of a nasal polyp which had been removed previously and reported as being of adamantine structure. Lemaitre, in the discussion which followed, agreed with this opinion that removal of the cyst and opening of the antrum would have sufficed. He states that as true adamantinomas are met with "in the lower jaw only," he regards the growth as a degeneration of a "paradental cyst."

Bloodgood further affirms that even after a period of 29 years, if not subjected to previous operative interference, these growths will yield to radical operation. In the upper jaw, however, the early involvement of important structures makes adequate early intervention essential. The case of Santy²³ is one of considerable interest in that a solid growth of the lower jaw recurred in cystic form 6 years after resection of the jaw. Even then, no adenopathy was present.

Solid growths may grow as fast or as slowly as cystic tumors. In the congenital cases of Coote²⁴ and Massin,²⁵ both children were seen at the age of 6 months, at which time one of the solid growths in the latter's case had grown to a diameter of 1 inch. The cysts in Coote's patient, which were accompanied by suppuration, were apparently of appreciable size. Gentsch²⁶ asserts that cystic degeneration, which he believes is due to defective nutrition, is induced by pressure upon the tumor and points out that in his own case the solid part of the growth was that over the face. He explains the apparently rapid increase in size of the cystic type as due to their relative bulkiness. With Siegmund,²⁷ Jäger,²⁸ Hammer,²⁹ Papayannou³⁰ and Séneque,³¹ Gentsch believes that the connective tissue also takes part in the cyst formation. In the author's case also this appears to be true.

According to Kronfeld³² the presence of osteoclasts in the stroma indicates rapid resorption of bone, and so ready extension of the tumor. This is reported in the case of Chibret,³³ the epular case of Böhmig,³⁴ and that of Bozo and Lattes.³⁵ Of the 25 upper jaw cases reviewed by Gentsch 14 were described as cystic, 10 as solid, and 1 as questionably solid, showing that cystic growths are as common in the upper jaw as are solid tumors. Of a total of 110 cases of tumor of the upper jaw reviewed, the author found that 60 were stated to be cystic and 40 solid. Solid tumors of the lower jaw occur also, although less frequently, but many cystic cases, such as those described by Carter,³⁶ are reported as being partly solid. The

tumors reported by Nové-Josserand and Bérard,³⁷ St. Germain,³⁸ Derujinsky,³⁹ Nasse,⁴⁰ Chibret,³³ and Kruse⁴¹ were all solid growths occurring in the lower jaw.

There is little doubt that solid growths are more likely to be treated by radical operation in the first instance, whereas cysts are often inadequately curetted, which probably explains Nové-Josserand's assertion that solid growths do not recur. There appears to be no constant relation between the consistence of these growths and their histology.

The histology of recurrences of these tumors also varies greatly. "Enamel" was present in a third recurrence of the polycystic type showing differentiated histology in D'Aunoy and Zoeller's⁴² third case, while in the first case of Simmons, already quoted, the recurrence consisted entirely of undifferentiated cells. In the cases reported by Falkson,⁵ Becker,⁴³ and Wright,⁴⁴ the recurrences showed differentiated histology. In the tumor of the upper jaw reported by Cordeiro and Cansanco,⁴⁵ after 15 years duration and frequent incisions the histology persisted in the differentiated form. There would appear to be evidence showing that it is possible for adamantinomas to develop malignant characteristics associated with three types of metaplastic change in the growths.

Stromal changes of a sarcomatous type have been reported by Eve,⁴⁶ in a tumor of the lower jaw in which enamel organ tissue and epithelium were present also. Fergusson's case, reported by Heath,⁴⁷ was that of a round cell sarcoma with metastases to the pelvis and biceps, after the cystic growth had lasted for 35 years. He described also⁴⁸ a case of adamantinoma showing this change. There were no enlarged glands.

The Report on Odontomes states that there is on record another case in a boy of 5 years, in which the dentition was complete. The growth contained the crown of a permanent third molar and the tissue resembled that of a sarcoma, and also "was not unlike dentin." All these growths occurred in the lower jaw. Mainguy's case⁴⁹ is probably also of this type, as he states that the growth seemed to him to arise in connection with the second left upper molar tooth, although the tissue was shown to be sarcomatous.

Epithelial changes apparently may be of two types, as might be expected from the nature and origin of these growths: (1) cylindromatous metaplasia, *i.e.* of the basal cell type alone, as reported

by Kaufmann,⁵⁰ Bozo and Lattes,³⁵ Bercher and Grandclaude,⁵¹ Gernez and Surmont,⁵² and others. In the case of Bercher and Grandclaude there was sudden increase in the rate of growth, with fracture of the bony wall associated with cylindromatous changes in the tumor. Raach,⁵³ in Case 3, describes a similar change, and Simmons²¹ also, in a woman of 62 years. Séneque describes a tumor with a "cylindromatous stroma." (2) Squamous changes of a carcinomatous character occurring in growths of the epidermoid or acanthomatous type are reported by Suker⁵⁴ and Ewing.⁵⁵

The sarcomatous changes in the stroma have been held responsible for the metastatic deposits reported by Eve,⁴⁶ Fergusson,⁴⁷ and Hutchinson,⁵⁶ while in Ewing's first case the metastases were definitely histologically related to the primary fibro-epithelial tumor in the jaw. In the second case, after the fourth recurrence, metastases to the lungs, neck and cervical lymph glands occurred. Simmons²¹ also reports 2 cases in which metastasis occurred; in one the growth was of 14 years duration, and in the other 12 years. In Case 7 the metastatic deposit exhibited the same histology as the primary tumor, while in Case 1 it showed a more differentiated histology. Bernays⁵⁷ believes that constitutional weakness induces a malignant condition. In all these cases there was a long history with record of frequent operative interference.

The case of Vorzheimer and Perla,⁵⁸ in which there was removed postmortem from the right bronchus a mass consisting of tumor tissue composed of cylindrical cells, a few stellate cells and central masses showing a roughly formed epithelial pearl structure with numerous spindle cells enclosed in a fibrillar network, is of interest as the authors stress the fact that there was no evidence of a malignant condition in the tissue. The patient was a man of 38 years, in whom an upper jaw adamantinoma had been present for 21 years and who had undergone several operations, with radium treatment also. Following this a radical operation was performed, but the patient developed lung complications and died. The authors are inclined to the belief, based on the histology of the mass in the bronchus and upon the fact that no invasion of the bronchial walls had taken place, that the lung involvement was due to aspiration of tumor tissue into the bronchus and not to metastasis of the growth.

The following report of a case of adamantinoma of the upper jaw, occurring in an Indian patient, is presented in view of the

comparative rarity of the condition and also because of the peculiar points in the histology of the growth.

REPORT OF CASE *

A portion of a partly solid growth was received for pathological examination on April 5, 1933, with the following history:

"The patient, Mohd. Hafiz, is a Moslem youth of 18, admitted as suffering from a hard swelling of the left upper jaw, duration 1 year. The tumour is situated over the second molar tooth, appears to have been painless, and is not tender. The teeth are regular; the jaw appears to be expanded. The jaw was X-rayed and the film suggested the presence of cystic formation in the region of the tumour.

The growth at operation was found to be partly solid and partly cystic; it had invaded the antrum. The wisdom tooth which had erupted into the antrum was surrounded by solid tumour tissue. The tooth and tumour tissue were removed and the cavity curetted."

Unfortunately, it has not been possible to obtain the subsequent history of this patient.

Macroscopic Appearance: The tumor tissue was of firm consistence, reddish in color, and its cut surface exhibited the presence of minute cyst-like spaces, which varied in size from being barely visible, to 0.6 cm. in diameter. The tissue was encapsulated.

Microscopic Examination: The upper part of the growth is surrounded by fibrous tissue which forms a rough but distinct capsule. This consists of comparatively well formed connective tissue bundles somewhat irregularly arranged and exhibiting in places well marked myxomatous degeneration, resulting in the development of cystic spaces varying from 10μ to a size visible to the naked eye in diameter. Some are empty and others contain mucoid material. The nuclei in this region are scanty, well formed and stain homogeneously. The blood vessels are numerous and possess definite walls. More or less linearly disposed in the outer margin of this part of the growth are many fragments of osteoid tissue exhibiting well marked lacunae but no Haversian canals. The lower part of the fibrous capsule of the tumor is made up of connective tissue of a more homogeneous myxomatous character and of lighter texture, with fewer and less well formed vessels, which are mainly distributed along its outer margin. Here, too, an almost continuous boundary is formed by calcified material, similar to that mentioned above. In all instances there exists a narrow margin of firm connective tissue external to the

* This case is reported through the kind permission of Lt.-Col. Wilson, I. M. S., Civil Surgeon, Delhi.

calcified material. Figure 3 illustrates the general scheme of arrangement of the tumor.

The remainder of the growth consists of epithelium and cellular stroma present in varying proportions, and is irregularly lobulated (Fig. 4). In many places marked degeneration of the stroma has resulted in the formation of cystic spaces containing a delicate homogeneous acidophilic substance (A, Fig. 4). In other parts the spaces are empty, and elsewhere the stroma is of a highly cellular character, and here a degeneration of a different type appears to have taken place (B, Fig. 4). This material is granular, highly acidophilic, and in places where the stroma is in contact with the epithelium can be seen as irregular, rounded calcified masses composed of aggregations of similar granules. This substance differs from that shown in C, Figure 4, in that an eccentric prismatic structure is absent; it is of definitely granular character and exhibits greater affinity for the acid stain. It appears to be developed in and from the connective tissue only, but cannot definitely be interpreted as being poorly formed dentin. In one instance an entire cyst cavity was filled with this material, the granules varying in size but all more or less rounded in shape, while scattered along the margin of the cavity were larger masses of paler color, but of obviously the same origin. In places, it seemed that the material was being laid down on fine processes produced by change in the stroma.

The epithelial disposition is roughly lobular, the lobules varying in size and delimited by the highly cellular stroma. Many of the lobules are formed by masses of small epithelial cells of a roughly spherical shape, with scanty cytoplasm and large, somewhat pyknotic nuclei. No mitotic figures were seen, although in many instances the chromatin was diffuse. Alveolar distribution was also seen and the cells were of a low cylindrical type, regularly arranged on a basement membrane and flanked by one of two rows of flattened cells, thus reproducing the arrangement of cells of the inner and intermediate layers of the developing enamel organ. In several instances, without alteration in the peripheral row of cells, those of the central mass had begun to degenerate by vacuolization of the cytoplasm, so that the nuclei appeared to be connected by fine cytoplasmic processes only. In Figure 5 is seen a further development of this process. Here the marginal cells have assumed a roughly cylindrical form and are disposed at right angles to the basement membrane,

while the central mass of cells has undergone marked degeneration, resulting in the formation of stellate cells similar to those forming the enamel pulp in the 4 months fetus. It is noteworthy that these cells are in this case formed by degeneration of cells of the basocylindrical type and not of the malpighian type, as is seen in the developing enamel organ. Indeed, no malpighian cells were seen in any part of the tumor. In Figure 3 B an early stage of this degeneration is seen.

Further stages of evolution of the epithelial structure are seen in Figure 5 and at D in Figure 4. These consist in the development of regular alveolar spaces formed by a single or double row of high cylindrical cells with well staining homogeneous or slightly pyknotic nuclei disposed toward the cavity or lumen. At the outer margin can be detected a row of supporting flattened cells delimited by a well staining basement membrane. A distinct membrane lines the alveolus and can be seen clearly in Figure 4. This resembles the preformative membrane of the enamel organ, and is similar to that described by Siegmund²⁷ in a case of adamantinoma. Attached to the inner margin of this structure are fragments of a substance that appears to be produced by the epithelium and a mass of which lies free in the alveolar space (C, Fig. 4). From its structure it appears to be built up of successive layers of roughly hexagonal prisms which are highly irregular in size and distribution, having a vague resemblance to enamel.

At one point in the outer margin of the tumor an interesting development can be seen (Fig. 6). Here there is a narrow, finely moulded rim of material similar to that seen at C in Figure 4. Arranged over it is a well defined arch of a substance consisting of fine prisms closely arranged so as to give the appearance of canaliculi, with a central plaque of homogeneous calcified material. Covering this and extending outward into a somewhat uneven and indefinite margin is material of a similar character but of lighter texture. It is seen to be disposed over the substance shown at B in Figure 4. In no other part of the tumor are these substances seen in direct contact, and in all other instances the enamel-like material is contained within an alveolar space. In further support of the statement that the material shown at B in Figure 4 is formed solely in the connective tissue is the fact that in one place it definitely assumes the place of the connective tissue, which at that situation forms a

dense, membrane-like support to material seen at c in Figure 4, and which prior to assuming the calcified form is seen in bead-like formation arranged along the fibrous structure. Finally, a mass of material resembling in every detail that shown at B in Figure 4 is formed.

The structure of the growth leaves no doubt that this tumor is an adamantinoma containing calcified tissue of three types, whose characteristics are difficult to interpret absolutely from the structure exhibited. The disposition of the enclosed molar tooth suggests a gubernacular rather than a dental germ origin, as does also the irregular distribution of two of these calcified tissues (B and c, Fig. 4), while their presence in both true and reversed order (Figs. 4 and 6) is highly significant.

The only record which could be found of an adamantinoma in an Indian patient is that of Tirumurthi.⁵⁹ The tumor in this case, a female patient, was situated in the lower jaw and weighed 3 pounds at the time of operation.

In the Report on Odontomes the following figures are given:

Dental Cysts: Found equally in both sexes; a total of 18 records were found, with 50 per cent of these occurring in the upper jaw. In 5 of these upper jaw tumors the tooth involved is stated—incisor 1, molar 1, premolar 3.

Dentigerous Cysts: Eighty-four cases, of which in 76 the sex was stated; 39 occurred in women and 37 in men. Forty-one were upper jaw cases; 30 involved the canine teeth, 16 the incisor teeth, 13 the third molars, 12 the first or second molars and 11 the premolars.

Multilocular Cysts: They state that all of 39 cases were lower jaw tumors.

Gentsch collected 24 cases with tumors of the upper jaw from the literature and reported 1. Cordeiro and Cansancao collected 27 cases from the literature, and reported 1.

The following cases have been traced by the author:

Juxta- or Pararadicular Adamantinomas (Dental Cysts): Twenty-three cases, in 20 of which suppuration was a feature; in the remaining 3 the patient attributed the growth to trauma.

Simple Adamantinomas: Fifty cases.

Epular Adamantinomas: Weinlechner⁶⁰ 2, Böhmig²⁴ 1, Raach⁵³ Cases 2 and 3 (described as "mixed tumours of the palate"). Probably also the bilateral growths reported by Lantier⁶¹ were of this

nature. Wohl⁶² described an adamantinoma of the upper lip close to the junction with the gum. Moulonguet and de Lambert⁶³ offer evidence to show that congenital epuli are of the nature of adamantinomas.

Dentigerous Adamantinomas in which the Enclosed Tooth Was an Unerupted Tooth: In 12 cases a canine tooth alone; in 3 cases the wisdom tooth alone; in 3 cases an incisor tooth; in 3 cases the first or second molars; and in 3 the tooth is not named. The records of the cases reported by Wrede and by Ricke were not obtainable, but Gentsch refers to the former author as not stating whether the wisdom tooth had erupted or not, and in Wrede's case no report was made as to whether the tooth was an unerupted or supernumerary one.

The cases in which the canine tooth was enclosed are those of Cumston,³ Heidé,⁶⁴ Gurd,⁶⁵ Mayet,⁶⁶ Delie,⁶⁷ Broca,⁶ Nélaton,⁶⁸ Bayer,⁶⁹ Gensoul,⁷⁰ Crocquefer,⁷¹ 2, Chompret and Dechaume,⁷² 2.

The wisdom tooth was enclosed in 2 cases of New,⁷³ that of Tellier,⁷⁴ and in the author's case.

The incisors were found enclosed in the cases of Syme,⁷⁵ Salter,⁷⁶ and Vitalis.⁷⁷

The first or second molar was enclosed in the cases of Jourdain,⁷⁸ Ollier,⁷⁹ Lucas⁸⁰ and Jay.⁸¹ D'Aunoy and Zoeller⁴² do not report the tooth enclosed.

The following cases must be considered separately for various reasons:

Coleman⁸² reports a case in which a growth appeared at the age of 12 years, 28 dental corpuscles were present. Nine were single, each with a formed conical crown, 6 had many points, and there was present also an irregular dental mass. In the right upper jaw the canine premolar and first molar tooth were missing.

Tellaider⁸³ reports a case in a woman of 27 years, in the right upper jaw of whom were missing the first molar, both bicuspids and the canine tooth. One of these teeth erupted a year after the operation. At the age of 12 enlargement of the jaw commenced, later followed by infection. In the tumor were found 9 single teeth and 6 tooth masses with a covering of enamel.

The first case of Chompret and Dechaume, already cited.

The case of Banns,⁸⁴ in which an unerupted canine was present in one antrum and a molar tooth in the other.

Case 1 of Bayer, in which the growth contained 2 teeth, 1 of which was in the antrum. Both were canine teeth, one a temporary and the other a permanent tooth.

Dupuytren and Bransby Cooper are cited by Heath as each reporting a case of adamantinoma associated with an inverted tooth, but the tooth is not named.

The case of Morault,⁸⁵ in which a growth in the region of the left lateral incisor contained a malformed canine tooth astride the unerupted incisor, a condition which suggests that malunion of the halves of the canine tooth germ may account for such cases.

Many rudimentary teeth were present in the cases of Hildebrand,⁹² and of Gilmer⁸⁶; the former was in a child of 8 years, and the latter of 6 months duration in a boy of 14. In the former instance some 200 teeth were removed and in the latter 78.

In the case of Rousseau-Decelle and Crocquefer⁸⁷ the teeth numbered 15 and each was possessed of a separate pulp cavity.

Miller's⁸⁸ case is also of interest. In a child of 1½ years a growth of 8 months duration showed the presence of widely scattered immature permanent teeth.

In the case of Crocquefer (Case 2) in addition to the missing permanent canine tooth there was found in the tumor 1 normal tooth and 12 tiny teeth, surrounded by a pericorony sac.

In Crocquefer's first case, in a man of 60 years, the tumor extended from the absent canine to the maxillary tuberosity, involving the entire floor of the sinus. Two small granulomatous nodes on the floor of the sinus were shown to be adamantine in structure; they were separated from the cystic portion of the growth which was lined by epithelium.

In the case reported by Cordeiro and Cansancao⁴⁵ cement was found.

In the course of a discussion in connection with the report of cases by New,⁷³ Teter stated that he had seen several cases of multilocular cysts of the upper jaw, some containing enamel and cementum.

The case of Pedrescu-Rion,⁸⁹ in which enamel was present in a recurrence of an upper jaw growth, is of considerable interest, especially as the patient gave a 4 plus Wassermann reaction.

The case of Reverdin⁹⁰ and that of Jeannel,⁹ already cited, are apparently of the nature of solid acanthomas.

It is possible that better drainage afforded in cases of upper jaw sepsis has some influence in reducing the number of adamantinomas met with in the upper jaw, as there is no doubt that dental cysts occur at least as frequently there as in the lower jaw, and according to Gentsch and others, cystic development in these growths is facilitated by defective nutrition and pressure, both of which factors are closely related to infection. The fact that these tumors appear to be associated with unerupted teeth, equally in either jaw, supports this hypothesis also. Developmental defects, as affording mechanical interference with eruption, may play an important rôle in the incidence of adamantinomas connected with unerupted canine and incisor teeth, as suggested by Chaminade⁹¹ and Kegel.¹⁶ It has been shown that entirely solid tumors are rare in either jaw, but that in the upper jaw, while probably a higher proportion of more nearly solid tumors is met with than in the lower jaw, predominantly cystic growths are at least as frequent as the more solid variety.

CONCLUSIONS

1. There appears to be insufficient evidence on which to separate "dental cysts" and "dentigerous cysts" from the group of adamantinomas proper.
2. As the basal cells of the gingival epithelium of the fetus appear to be capable of evolution in two directions, either toward the formation of typical malpighian and stratified epithelium or toward "cylindrization" and enamel secretion, the growths of purely malpighian cells had better be separated from the group of adamantinomas; the term suggested for them is "paradental acanthoma."
3. Gross structure and histology cannot be regarded as an index of rate of growth, possibility of recurrence, or malignant changes in the adamantinoma.
4. Malignant metamorphosis in these tumors may be of three kinds: (1) sarcomatous change in the stroma; (2) cylindromatous; or (3) squamous carcinoma changes in the epithelium. This tends to bear out the theory of two distinct non-interchangeable forms of evolution of the basal cells.
5. There is some evidence to show that better drainage of septic dental conditions may be responsible for the greater rarity of adamantinomas in the upper jaw.

NOTE: I wish to thank Dr. Lester Kahn, who kindly lent his copy of the Report on Odontomes, and Dr. Anna Goldfeder, who very kindly supplied a translation of one of the articles in German.

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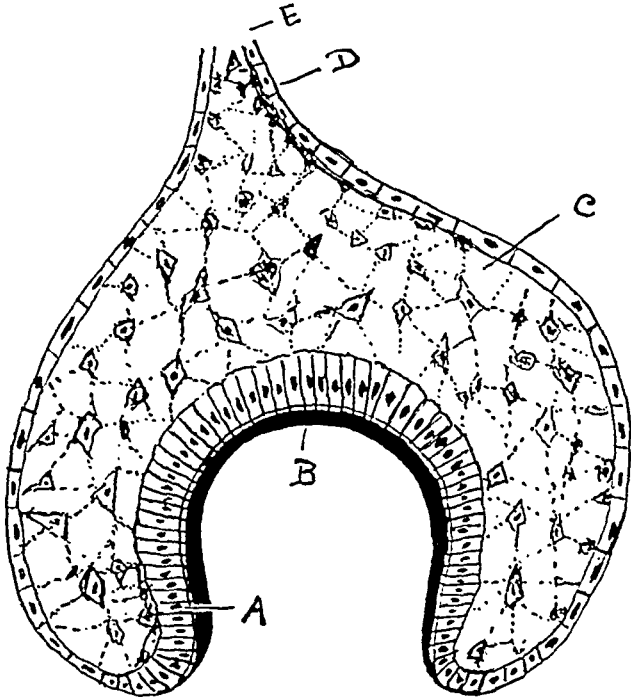
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DESCRIPTION OF PLATES

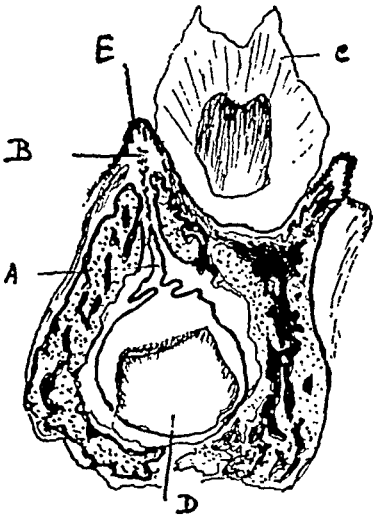
PLATE 172

FIG. 1. Diagram of the enamel organ at the 5th month. A = enamel-forming cells; B = enamel; C = stellate cells of the enamel pulp; D = external epithelial layer; E = cord of the enamel organ. The preformative membrane lies between A and B.

FIG. 2. A transverse section through the lower jaw passing through the temporary and permanent molars in a child of 3 years (Malassez and Galippe¹). A = iter dentis; B = gubernaculum dentis; C = temporary molar; D = permanent molar; E = gum margin.



1



2

PLATE 173

- FIG. 3. Illustrating the distribution of the stromal and epithelial elements of the tumor. A = osteoid tissue resembling cementum; B = formation of stellate cells.
- FIG. 4. Showing the various epithelial forms present in the growth. A = stromal degeneration, acidophilic in reaction; B = the substance formed in relation to connective tissue only suggestive of poorly formed dentine; C = the substance apparently derived from the epithelium and of roughly prismatic structure; D = the high cylindrical epithelial cells.



3



4

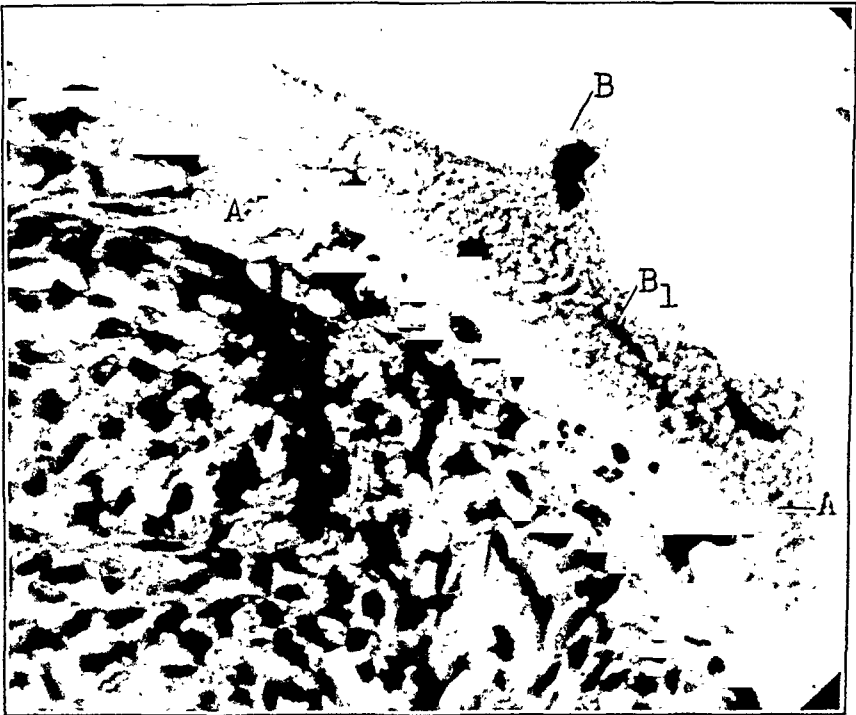
PLATE 174

FIG. 5. Showing the production of the substance shown at C in Fig. 4, by degeneration of the epithelium.

FIG. 6. The relation of the two types of calcified material at the margin of the growth, but within the capsule. A = the dentine-like substance; B = the finely prismatic structure; B₁ = a less well formed stage of the same substance.



5



6

CONGENITAL ATRESIA OF THE TRICUSPID ORIFICE AND
ANOMALOUS ORIGINS OF THE CORONARY ARTERIES
FROM THE PULMONARY ARTERY *

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A case of atresia of the tricuspid orifice with associated inter-ventricular septal defects and patent foramen ovale, and with anomalous origins of both coronary arteries from the pulmonary artery, has recently been observed in this laboratory. Primary atresia of the tricuspid orifice is an uncommon congenital cardiac malformation. Abbott ¹ mentions only 9 cases of this condition in a review of 850 cardiac malformations. In a more recent survey Breslich ² has recorded a total of 13. Two more cases have been reported ^{3, 4} since then which, together with the present one, brings the total to 16. The origin of one coronary artery from the pulmonary artery has been reported ^{5, 6, 7} by several observers. A survey of the literature, however, yielded no instance where both coronary arteries arose from the pulmonary artery. In view of the rarity of the condition the following case is reported.

REPORT OF CASE

Clinical History: The patient, a white female infant, 10 hours old, was born in the New Haven Hospital on Jan. 18, 1934. The mother, a 26 year old multipara, entered the hospital 1 month before the expected date of delivery because of vaginal bleeding. The condition was diagnosed as placenta marginalis and a Voorhees' bag was inserted to control the bleeding. The child was delivered spontaneously 3 hours later at 10.00 P.M. The child cried and breathed spontaneously and, except for moderate cyanosis and low temperature, appeared to be in good condition. It was seen on several occasions during the night and appeared to be doing well. When seen at 7.00 A.M. on the following day it was markedly cyanotic and respirations had ceased.

At autopsy the child was well nourished and well developed. It appeared to be full term and weighed 2725 gm. The mucous membranes and the skin of the head, face and neck were deep purple. The

* Received for publication May 22, 1934.

significant anatomical findings, in addition to the malformations in the heart, were bilateral congenital pulmonary atelectasis and hemorrhage into the tentorium cerebelli.

DESCRIPTION OF HEART

The heart *in situ* was globular and did not appear enlarged; its transverse diameter was 5 cm. and that of the chest at the same level was 8.5 cm.

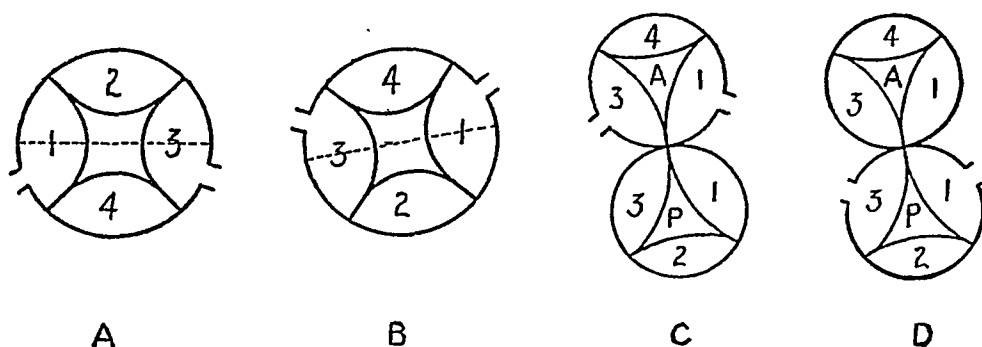
The pulmonary artery and aorta arose in the usual manner but showed a striking disproportion in their relative sizes. The former appeared to be about one-third the size of the aorta, which was of average caliber. One cm. from its origin the pulmonary artery divided into two vessels of equal caliber, each 1 mm. in diameter, one of which joined the aorta as the patent ductus arteriosus and the other continued as the left pulmonary artery. The aorta pursued its usual course but gave off from its ascending portion 1 cm. from the aortic ring a vessel 5 mm. in diameter which entered the hilum of the right lung. The vessels supplying the head and neck arose from the arch of the aorta in the usual manner. The distribution of the superior and inferior venae cavae appeared normal.

The heart weighed 19 gm. The apex was rounded and was composed entirely of the left ventricle. The right atrium was not dilated. The muscoli pectinati were well rounded and the wall was not thickened. The foramen ovale, which measured 1 cm. in diameter, was covered by a membranous fold of endocardium, except for a slit-like opening 2 mm. wide along the anterior margin. The coronary sinus opened into the right atrium at the usual site through an orifice 4 mm. in diameter. There was complete absence of the atrioventricular orifice. A pit-like depression in the thick muscular septum between the right auricle and ventricle marked the site of the tricuspid valve. No vestiges of the valve cusps were present. The right ventricle was an aplastic structure whose wall measured 1 mm. in thickness. Two circular defects, each 2 mm. in diameter, were present in the interventricular septum. One was situated in the membranous portion, and the other about 8 mm. directly below it in the muscular portion. The pulmonary artery arose from the conus arteriosus as a thin-walled vessel whose circumference was 1 cm. The three semilunar cusps were thin and delicate. The coronary arteries arose from the sinuses of Valsalva behind the two posterior cusps, the right cor-

onary from the right posterior sinus, and the left coronary from the left posterior sinus. The subsequent course and distribution of each of these vessels was normal. The left atrium was not dilated and its wall was not thickened. The only unusual feature observed here was an anomalous communication with the coronary sinus through a circular orifice 4 mm. in diameter situated on the posterior wall 5 mm. to the left of the foramen ovale. The left atrioventricular orifice measured 3.8 cm. in circumference. The leaflets of the mitral valve were thin, delicate and well formed. The left ventricle was markedly dilated and hypertrophied; its wall measured 4 mm. in thickness. The aorta arose from the ventricle as a large, well formed vessel which measured 2.4 cm. in circumference. The aortic valve was composed of three thin, delicate, semilunar cusps. No orifices were present in the sinuses of Valsalva behind any of these cusps.

DISCUSSION

The relation of congenital atresia of the tricuspid orifice to abnormalities in the embryological development of the septa of the heart has been adequately described by Breslich,² who also has reviewed the literature on this subject.



TEXT-FIGURE 1

Diagram showing the position of the distal bulbar swellings and coronary orifices before rotation, after rotation, and after division to form the pulmonary artery and aorta in the normal heart (A, B, C). D shows the arrangement of the coronary orifices in the present case. Modified after Feller.

The mechanism of the normal development of the pulmonary artery, aorta and coronary arteries is well presented by Feller,⁷ the chief steps of which are shown in the line drawings in Text-figure 1. The truncus arteriosus with the four distal bulbar swellings and with the origins of the coronary arteries from 1 and 3 are shown in A. The

positions of these bulbar swellings after rotation through approximately 180° has occurred are shown in B. The subsequent fusion and division of 1 and 3 to form the pulmonary artery and aorta are shown in C. Thus, the coronary arteries normally arise in the forward half of the bulbar pockets 1 and 3, close to 4, which portions subsequently form the anterior cusps of the aortic valve. Should the coronary artery orifices instead arise in the posterior halves of 1 and 3, close to 2, which subsequently form the posterior cusps of the pulmonary artery, they would have the positions indicated in D, which apparently occurred in the present case.

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DESCRIPTION OF PLATE

PLATE 175

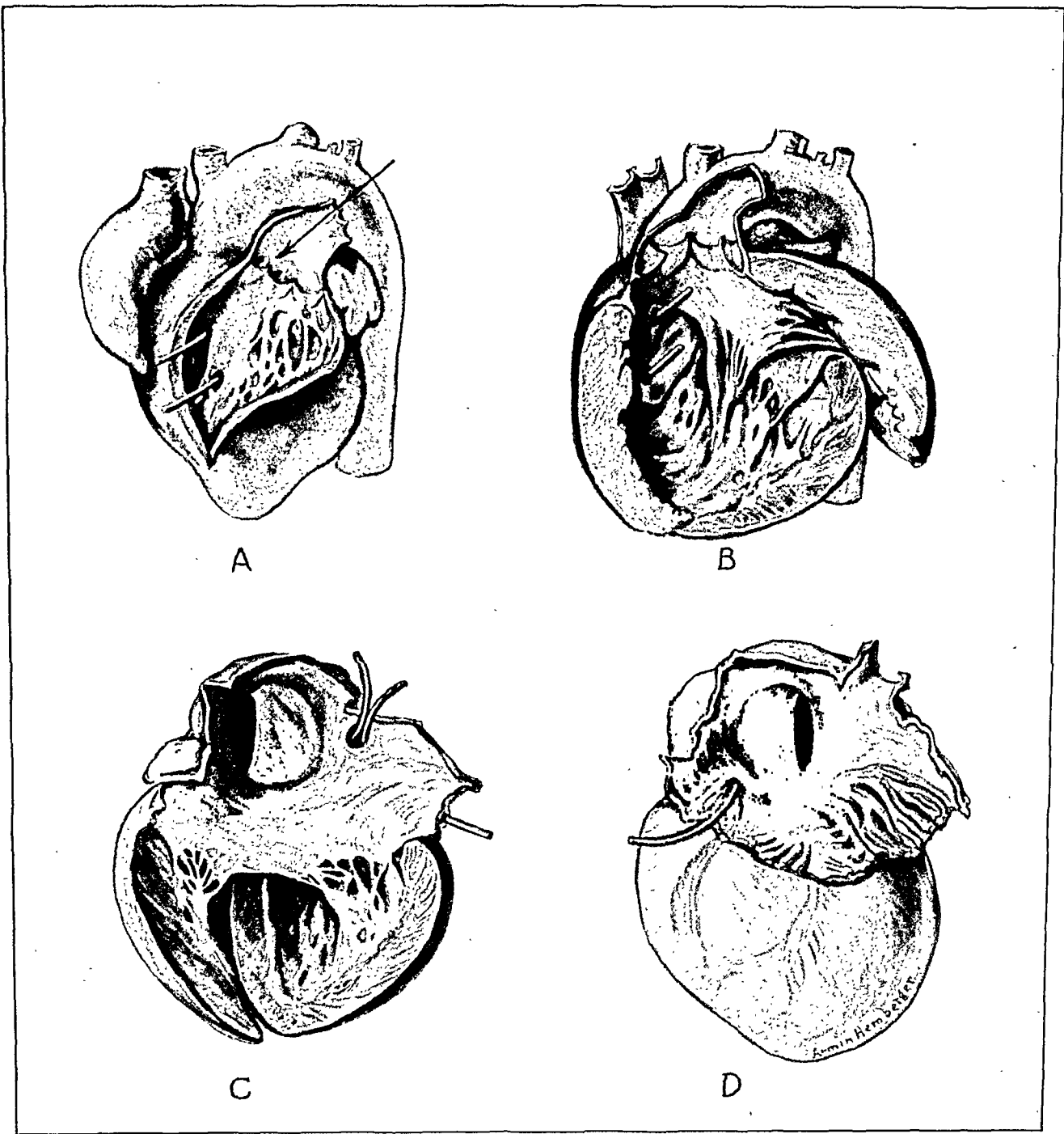
FIG. 1. (A 3038.) Atresia of tricuspid valve, with patent foramen ovale and interventricular septal defects; coronary arteries arising from pulmonary artery.

A = Right ventricle and pulmonary artery. Probes through septal defects. Orifices of coronary arteries in pulmonary artery. Aplastic right ventricle.

B = Left ventricle and aorta. Probes through septal defects. No coronary orifices in aorta.

C = Left auricle and left ventricle. Patent foramen ovale. Anomalous opening of coronary sinus.

D = Right auricle. Patent foramen ovale. Orifice of coronary sinus. Absence of tricuspid valve.



I

CALCIFICATION IN THE BRAINS OF EQUIDAE AND OF BOVIDAE *

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In 1926¹ I estimated the frequency of so-called calcification in the vessels of the anterior half of the globus pallidus in man, and of "calcified" degeneration bodies there and in surrounding tissues. The condition had of course been described previously, but usually in connection with specific maladies, notably paralysis agitans and encephalitis lethargica; it now appeared that it must be considered as a phenomenon more or less normal in advancing years. Ostertag² has since recorded similar observations. In other territories of the central nervous system "calcification" occurs much less often, almost always only in connection with local pathological conditions; the most marked examples are perhaps to be found in some cases of porrocephaly resultant from intra-uterine disease. Calcified "corpora amylacea" in the meninges are, however, of more or less normal occurrence.

While the mineral matter reacted strongly for iron, no definite evidence of the presence of calcium salts was forthcoming. Deposits of iron can give or obscure many of the color reactions commonly ascribed to calcium. Spatz³ referred to the deposits as "Pseudokalk." Cameron⁴ has studied very fully the staining properties of calcium salts and emphasizes the ease with which small amounts of iron are adsorbed from impure reagents, and so on; the globus pallidus is extremely rich in "free" iron (Spatz³) which might conceivably be concentrated in the affected vessels during life. At the time of my earlier publication I was not aware of the purpurin test for calcium, shortly afterwards used by Da Fano and Perdrau.⁵

This degeneration of the vessels of the globus pallidus is not confined to man. I have encountered somewhat similar appearances in even young monkeys (*Macacus rhesus*).⁶ On the other hand, I have

* Received for publication June 5, 1934.

never done so in rabbits, guinea pigs, mice or rats. As the ensuing account will show, calcification in various forms occurs with surprising frequency in the equidae, and also in cattle.

CALCIFICATION IN THE GLOBUS PALLIDUS

The brains of 16 horses, a Shetland pony and a mule were examined; none of the animals had a history of any illness other than the acute one to which it succumbed. Three under 7 years of age showed no calcification. Three (including the Shetland pony) aged 9, 9, and 10 years, respectively, exhibited calcified vessels in the globus pallidus, while one aged 11 years did not. The remaining 11 (including the mule) of ages between 13 and 25 years were all affected.

The histological appearances were in every way comparable with those in man. In different cases the deposits lay in the media of the vessels, in the adventitia, or in both. In some instances, relatively more frequent than in man, the affected vessels were almost obliterated by intimal thickening (Fig. 1), and the newly formed tissue also was on occasion lightly impregnated; these horses did not suffer from generalized vascular disease. A variable number of impregnated degeneration bodies were distributed similar to those in human cases.

In the brains of 2 of 7 cows (ages unknown) similar lesions were seen in the globus pallidus.

CALCIFICATION IN OTHER PARTS OF THE BRAIN

Two horses, aged 9 and 16 years respectively, exhibited finely granular deposits in the nervous tissue immediately adjacent to the adventitia of a few of the cerebral arteries. In one animal a half-dozen or so of the vessels in one part of the centrum semi-ovale were so affected; in the other two of the pontine vessels suffered. The mineral salts surrounded only a small part of the circumference of the vessels.

One horse, aged more than 20 years, showed most extensive lesions in the dentate nuclei of the cerebellum. Every vessel was heavily calcified and the tissues were strewn with calcified globules of all sizes (Fig. 2). Some of the capillaries were completely encased in sheaths of similar nature; considerable new formation of small vessels was apparent, and a degeneration of the nervous tissues with great reduction in the number of nerve cells. One large vessel had

apparently suffered thrombosis, with later recanalization; part of the artery wall and the remains of the clot were heavily impregnated with mineral salts (Fig. 3). These lesions possibly represented the aftermath of an acute process of unknown date and origin; the history of the horse prior to its fatal acute illness was not available.

The meninges and choroid plexuses of old horses frequently contain "corpora amylacea" which may or may not be calcified. In both young and older animals, in the former in the absence of changes in the globus pallidus, the larger meningeal arteries frequently show small calcified bodies lying usually immediately beneath the endothelium (Fig. 4). These corpuscles commonly consist of a larger laminated central body, roughly spherical or ovoid in shape, to which are attached several small outgrowths; their appearance may aptly be compared with that of a tortoise.

STAINING REACTIONS OF THE DEPOSITS

The deposits are blackened by the von Kossa method for detecting phosphates. They stain deeply with hematoxylin, purple or black according to the particular method employed. Tests for iron (Prussian blue reaction, Macallum's hematoxylin) are positive. Purpurin colors the degenerated areas purple, the color imparted to the dye when precipitated in the presence of iron salts. One hours immersion in 10 per cent oxalic acid prevents staining by the von Kossa or Prussian blue techniques; the areas now color red with purpurin, a reaction abolished by immersion in 5 per cent hydrochloric acid.

Thus, evidence of the existence of both calcium and iron salts in these deposits is forthcoming.

SUMMARY AND CONCLUSIONS

Calcification of the vessels of the globus pallidus is at least as frequent in middle-aged and old horses as in man at a corresponding period of life; it also occurs in cattle. In neither species can it be correlated with the pathological condition responsible for death. Since similar appearances are met with in monkeys, it seems probable that it may represent a biological phenomenon of some constancy in advancing life in the higher mammals. Unlike man, many horses, both young and old, show small calcified bodies in the intima of the larger

meningeal arteries. Calcification may sometimes be present in other parts of the central nervous system. The use of the purpurin test following treatment of sections with oxalic acid permits recognition of calcium salts in the presence of iron compounds; in the horse both are represented in the degenerated vessels.

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DESCRIPTION OF PLATES

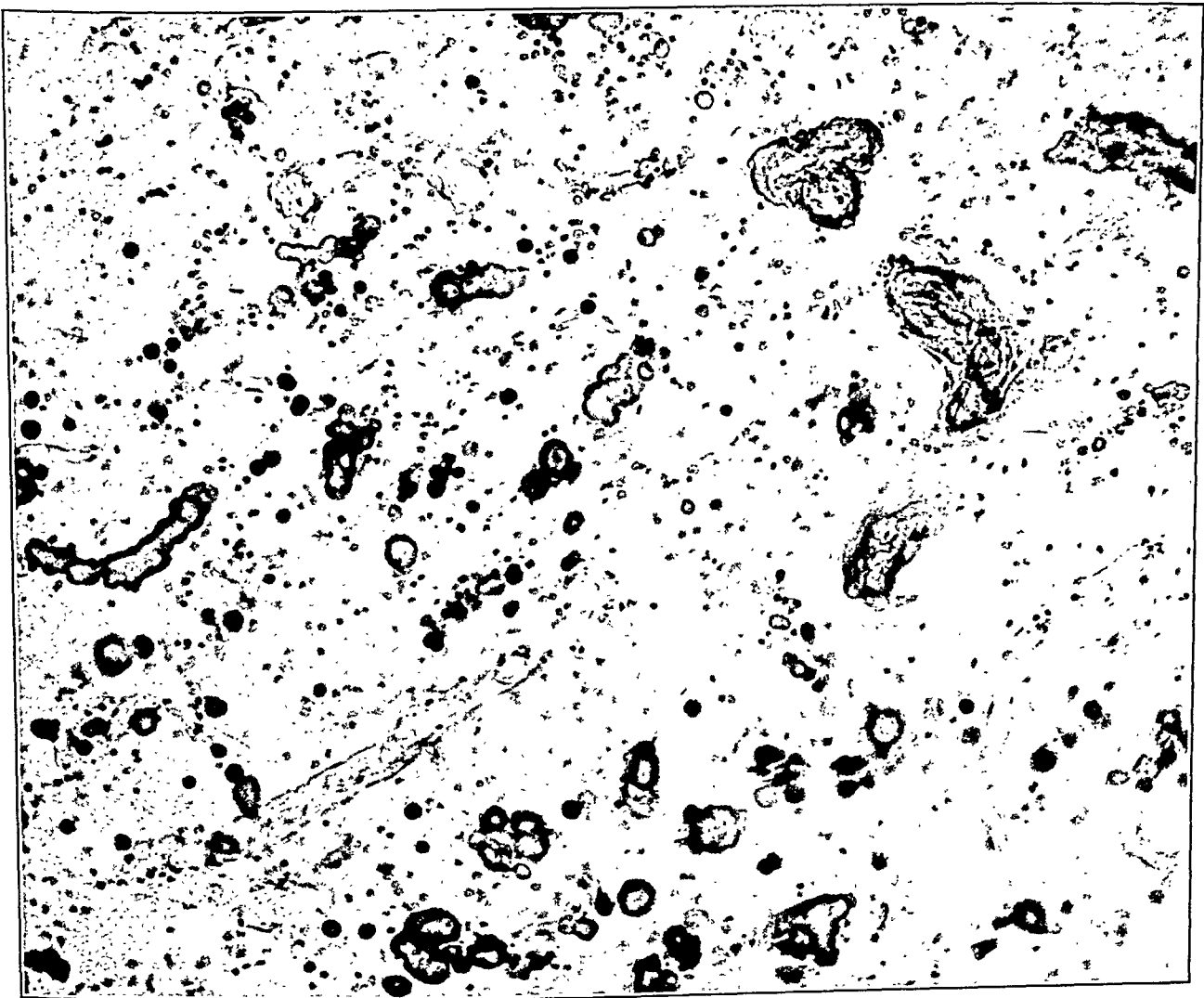
PLATE 176

FIG. 1. Calcified vessel of the globus pallidus of a horse dying of equine encephalomyelitis, showing marked thickening of the intima. Iron alum hematoxylin and Van Gieson's stain. $\times 340$.

FIG. 2. Vascular calcification and calcified globules in the dentate nucleus of the cerebellum in a horse dying of acute epizootic leucoencephalitis (MacCallum and Buckley). Iron alum hematoxylin and Van Gieson's stain. $\times 215$.



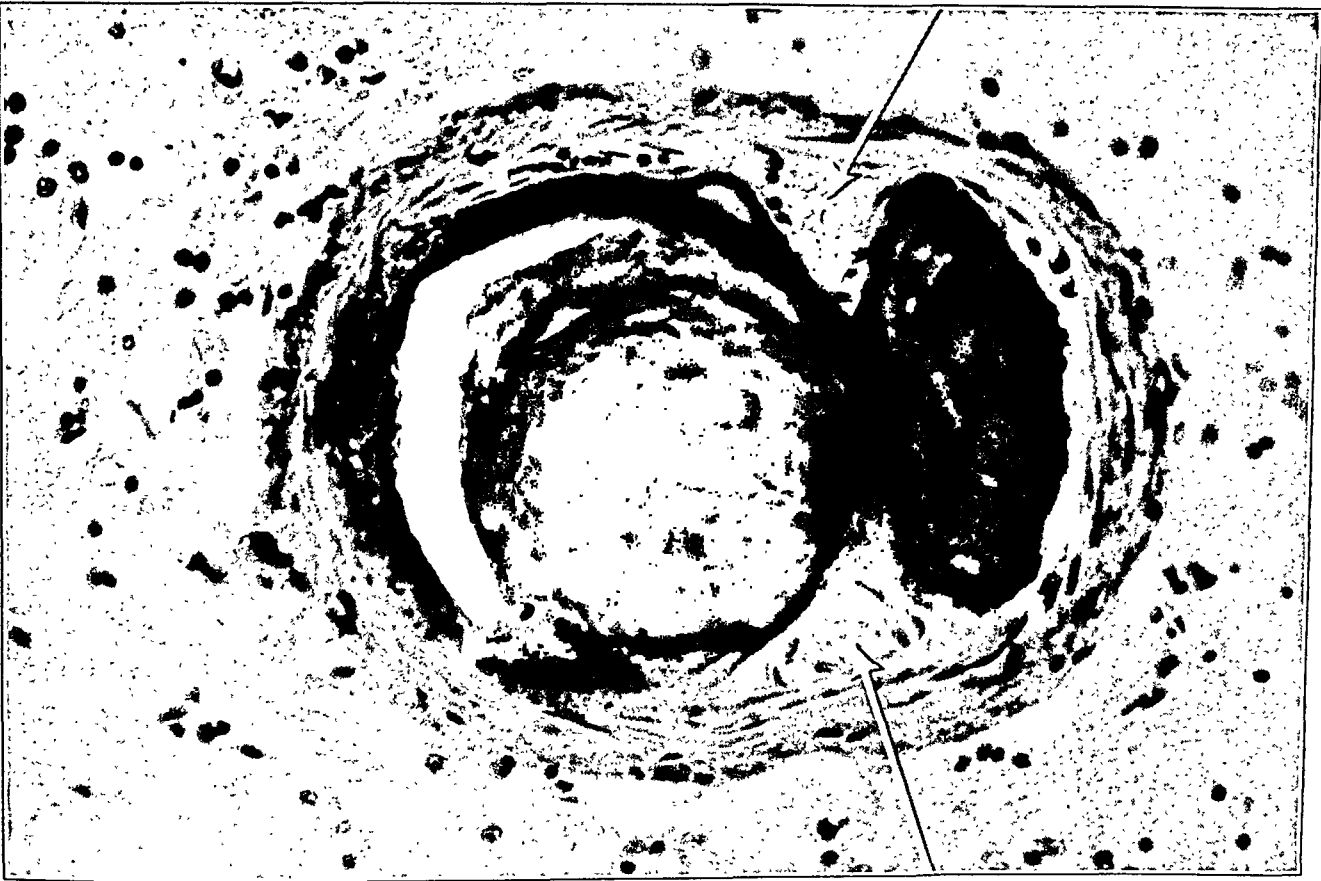
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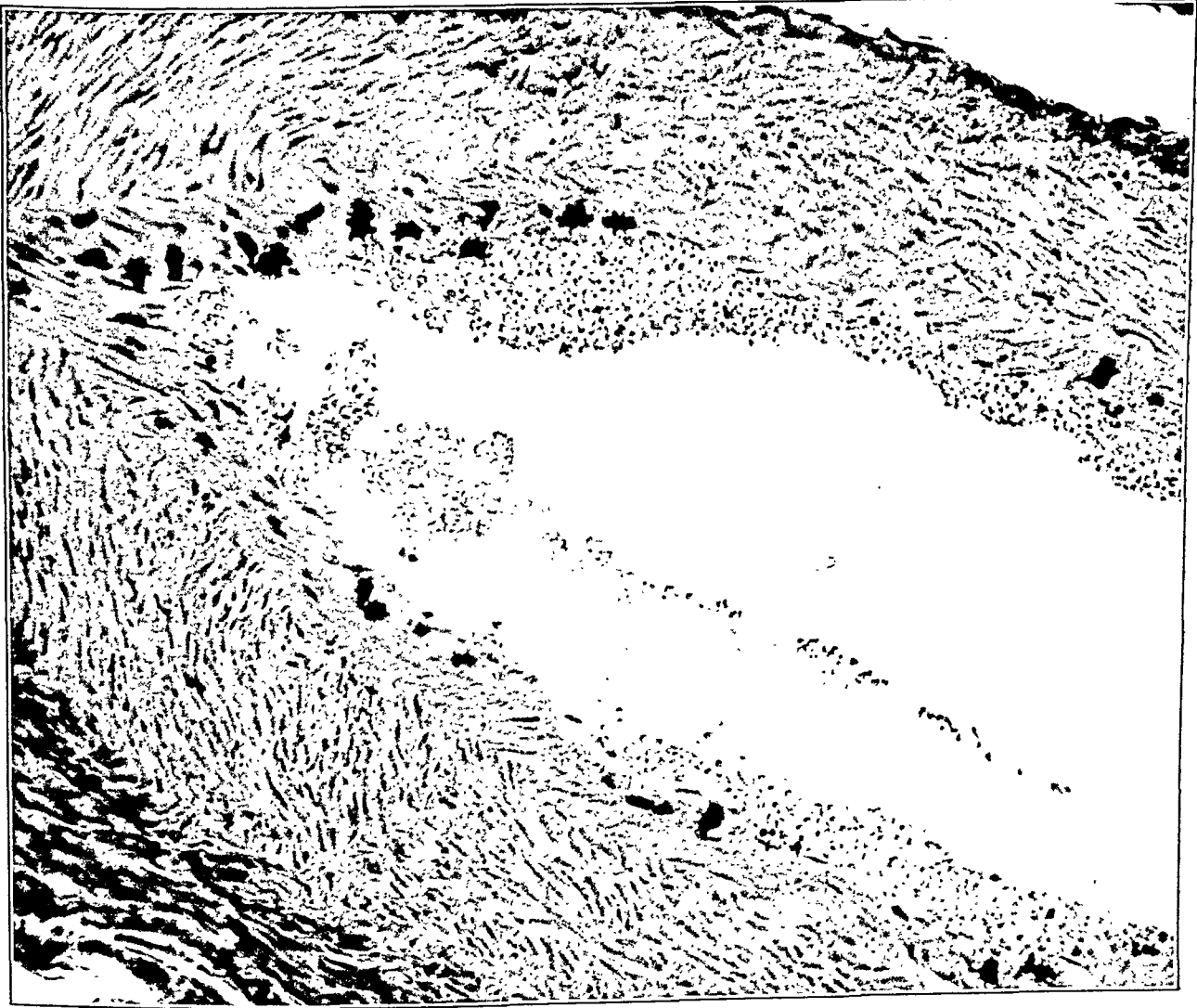
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PLATE 177

- FIG. 3. Calcified thrombus in a vessel of the dentate nucleus of the cerebellum; recanalization of the clot indicated by arrows. Same case as Fig. 2. Iron alum hematoxylin and Van Gieson's stain. $\times 365$.
- FIG. 4. Calcified bodies in the intima of a meningeal artery from a horse dying of forage poisoning. Iron alum hematoxylin and Van Gieson's stain. $\times 260$.



3



4

FOCAL FAT INFILTRATION IN THE LIVER *

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The statement that lipoma occurs in the liver is made in such textbooks as those of Borst,¹ MacFarland,² Adami and Nicholls,³ Ribbert,⁴ Stengel and Fox,⁵ Delafield and Prudden⁶ and Karsner,⁷ but no mention is made by Schridde,⁸ Ewing⁹ and others. A critical search of the literature, however, fails to reveal any case or report that can be indubitably accepted as a true case of lipoma of the liver. Rolleston¹⁰ stated that genuine fatty tumors do not occur in the liver and that what have been mistaken for these are small appendices epiploicae which have become detached from the colon and have come to rest between the diaphragm and the convexity of the liver. These are flush with the surface of the organ, but upon careful examination are found to lie outside the capsule of the liver. Turnbull and Worthington¹¹ described areas of atypical liver tissue under the capsule which are prone to fatty change, probably congenital anomalies. These changes, however, do not represent the size or appearance of the lesion to be described.

Because of the fact that there appears to be some doubt about the actual existence of lipoma of the liver and because the lesion about to be reported was first thought to be a lipoma, the following case is reported.

REPORT OF CASE

The patient, a 70 year old white female, died after surgical drainage of the gall-bladder. Autopsy revealed a scirrhus adenocarcinoma of the gall-bladder with local metastases to the liver, head of the pancreas and regional lymph nodes.

The liver weighed 1300 gm. The left lobe was relatively smaller than normal. The capsule was smooth and transparent and the edges were rounded. The color was normal. On the superior surface of the left lobe of the liver and directly under the capsule was a spherical nodule measuring 2 cm. in diameter. This was yellowish

* Received for publication June 6, 1934.

white in color, slightly raised above the surface, firm in consistence and not attached to the diaphragm. On section it greased the knife and on the cut surface the usual liver architecture was absent. The lesion was sharply demarcated from the surrounding normal liver as though it were enclosed within a capsule.

MICROSCOPIC EXAMINATION

A low power photomicrograph of the lesion (Fig. 1) stained with Mallory's aniline blue shows the outline to be somewhat irregular. There is no capsule. The only places where connective tissue can be recognized along the periphery are where the fat-like tissue abuts upon a portal space. Sections stained with sudan III reveal the fat to be of the neutral type.

Figure 2 shows the edge of the lesion in relation to the normal liver tissue. Here the infiltrating fat-like tissue can be seen in various portions of a lobule and again the absence of a capsule is apparent. The fat-like cells are of the adult "signet-ring" type and there is a suggestion of compression of adjacent liver tissue. The liver cells themselves show granularity of the cytoplasm but the nuclei are normal. There is a zone between normal and signet-ring cells in which liver cells contain small fat droplets. Along the periphery of the lesion and most particularly in abutting portal spaces there are occasional monocytes and lymphocytes. These cells are not present within the central portions of the lesion.

Within the center of the fatty lesion, but more clearly and with greater frequency along the periphery, small, intact bile ducts are present surrounded by a small amount of fibrous connective tissue and the cells containing fat.

Within the fatty area a moderate number of fairly large round cells with foamy cytoplasm and ovoid, deeply chromatic nuclei is found in the interstices between the liver cells infiltrated with fat. It is not known whether these cells represent young liver cells infiltrated with fat, or fixed or wandering histiocytes. The lesion is fairly well vascularized by large arteries and thin-walled veins and capillaries that appear to spring from the periphery. The arteries along the periphery, which seem to be derived from the portal spaces, show slight thickening of their walls and the veins for the most part are wide, thin-walled and gaping, although an occasional vein has a thickened wall.

DISCUSSION

In 1925 Huguenin,¹² as quoted by Hanser,¹³ described two types of focal fatty metamorphosis in the liver which had not previously been found in the literature.

Huguenin described one type of focal fatty metamorphosis occurring in the liver of healthy men and dogs and another type in men and animals dead of acute infectious disease. This latter type is found in the liver that is the seat of cloudy swelling.

The first type is found on the surface, is sharply delimited, although irregular in contour, and the diameter on the surface is at most 3 cm. and on the cut surface, at most, 1.5 cm. in diameter. Microscopic examination of the peripheral zones proves absolutely that the fatty metamorphosis is delimited by the borders of a lobule and that an individual lobule either is completely changed or not at all. The cells contain neutral fat. Further, there are changes in the vessels either within or without the focus and there is no evidence of inflammation, pigmentation or nuclear changes.

The second type is seen at autopsy in men and animals dead of infectious disease. Grossly these lesions appear exactly like the former type but microscopically show that the fatty metamorphosis is not contained within the borders of a lobule. Changes in the nuclei may be found and frequently lymphocytes are found between fat infiltrated cells. Huguenin believed that this latter type does not go on to complete recovery because of the nuclear changes and infiltration of lymphocytes, and that this type may end in circumscribed areas of cirrhosis.

Of the two groups, the lesion here reported should be placed in the second, although there are no significant nuclear changes and no infiltrations of lymphocytes within the fatty mass.

The lesion does not correspond to the usual picture of lipoma in that there is no trace of capsule and the supporting tissue is made up in considerable part of remnants of the capsule of Glisson. There are no features indicative of malignant neoplasm.

That the condition is a localized fat infiltration is supported by the fact that perilobular structures including bile ducts are found within the mass. There is no positive indication of marginal compression and between the normal liver cells and the fatty mass there is a zone, interpreted as transitional, in which many liver cells show

small fat droplets. The process differs from ordinary fat infiltration of the liver in that all the cells within the mass show almost complete distention of cytoplasm by a single, large fat globule.

SUMMARY AND CONCLUSIONS

A case of localized fat infiltration of the liver resembling lipoma in the gross and not identical with any similar lesion reported in the literature is reported. It is possible that other cases thought to be lipoma of the liver are of the same nature.

NOTE: The author wishes to express his thanks to Professor H. T. Karsner for the photographs.

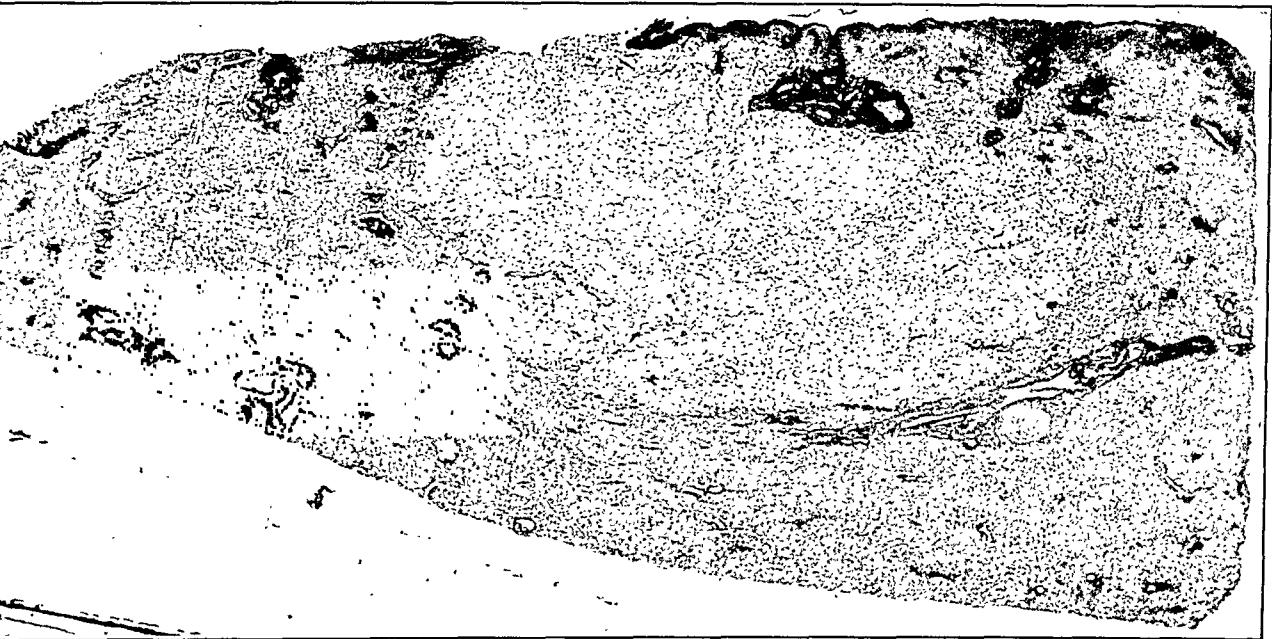
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DESCRIPTION OF PLATE

PLATE 178

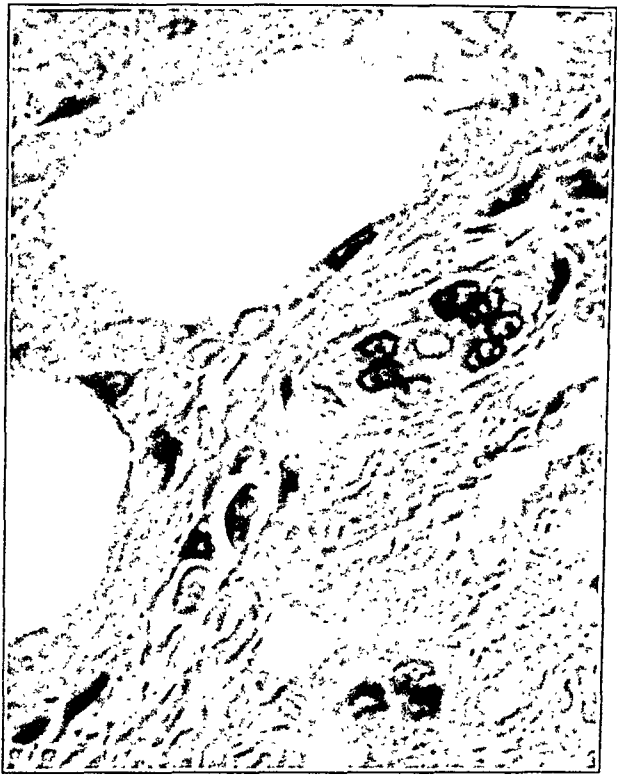
- FIG. 1. Section showing irregular contour of the lesion. Mallory's aniline blue stain. $\times 3$.
- FIG. 2. Section showing lack of sharp definition of the lesion and lack of limitation to a single lobule. Hematoxylin and eosin stain. $\times 150$.
- FIG. 3. Small bile duct near center of main mass. Hematoxylin and eosin stain. $\times 820$.



I



2



3

MENINGIOMA OF THE TUBERCULUM SELLAE WITH HYPEROSTOSIS *

REPORT OF A CASE WITH AUTOPSY FINDINGS

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Chicago, Ill.)

The clinical syndrome produced by meningioma of the tuberculum sellae has been well formulated in the contributions of Holmes and Sargent,¹ who reported 10 cases, and of Cushing and Eisenhardt² with 15 cases. The typical course is described by Cushing and Eisenhardt as gradual impairment of vision in a person of middle age with bitemporal constriction of the visual fields, usually not equal in the two eyes, and primary optic atrophy. In the early stages these tumors do not deform the sella or cause secondary symptoms of hypopituitarism.

While about one-fourth of meningiomas situated under the vault of the skull produce thickening of the adjacent bone, in none of the cases of suprasellar meningiomas described by the above authors was hyperostosis detected. In one case Cushing and Eisenhardt² found a slight invasion of the tuberculum sellae by tumor cells. We present the following case of meningioma arising over the tuberculum sellae in which a marked involvement of the underlying bone occurred.

REPORT OF CASE

Clinical History: T. B., No. 64972, male, a 42 year old painter of Denver, Colorado, was admitted to the University of Chicago Clinics on July 31, 1932, complaining of loss of vision of 15 years duration. His past and family histories, so far as could be determined, were irrelevant to the present illness.

The patient first noted impairment of vision of the left eye in 1917, which grew progressively worse until it was reduced to light perception in 1920. Lead poisoning was suspected in 1922. In 1928 the vision of the right eye began to fail but the patient was able to do his work until 1930, at which time the left eye became totally blind. By 1932 vision in the right eye was reduced to the perception of bright light. A history of temporal constriction of the visual fields could not be elicited. There had been no headache or alterations in weight or strength.

* Received for publication June 11, 1934.

Physical Examination: The patient was a well developed, obese male, weighing 81.7 kg. An abundance of hair was present over the chest, axillae and pubis. Sense of smell was intact. The left eye was totally blind, while light perception was present in the inferior nasal quadrant of the right visual field. Both optic discs were intensely white with clear-cut outlines. A large area of lamina cribrosa was visible in each. The pupils were about 6 mm. in diameter with reaction to light only directly in the right eye and only consensually on the left. Ocular movements were full with a coarse lateral nystagmus on looking to either left or right. The tongue protruded slightly to the left. Sensation and strength were everywhere intact. Except for a moderate exaggeration of the left knee jerk and ankle jerk all reflexes were normal. The basal metabolic rate was minus 16 per cent.

Laboratory Data: The blood and urine showed nothing abnormal. X-ray of the skull revealed enlargement of the sella turcica with partial decalcification of the posterior clinoids. In oblique views taken for the optic foramina the tuberculum sellae could be seen to be greatly thickened.

A diagnosis of meningioma of the tuberculum sellae was made and a right frontal osteoplastic flap was reflected. During the course of extirpation of the tumor with the Bovie knife a large artery was torn and the patient died from hemorrhage.

AUTOPSY REPORT

Postmortem examination revealed an intradural hemorrhage, slight generalized arteriosclerosis, early nodular hyperplasia of the prostate, fatty infiltration of the liver, pancreatic rests in the duodenal mucosa, submucous urachus rests in the urinary bladder and a bile duct adenoma in the liver, in addition to the intracranial tumor.

A large tumor mass 4.5 by 4.5 by 2.5 cm. lay on the undersurface of the brain in the midline. It extended from within 5 cm. of the frontal pole posteriorly to the tuber cinereum and markedly compressed the inferior surface of the brain and the medial surfaces of the temporal lobes (Fig. 1). The tumor was very hard, grayish in color, and showed calcium deposits on gross section. The optic chiasm was greatly flattened over the superior surface of the tumor. The left optic nerve was completely obscured and the right markedly compressed by extension of the tumor into the optic canals. The hypophysis was flattened and compressed into the posterior part of the sella turcica.

A roughened bony eminence 2.5 cm. long, 2.5 cm. wide and 5 to 7 mm. deep occupied the site of the tuberculum sellae and posterior portion of the cribriform plates. The entire sphenoid was removed as a specimen. Section of this specimen in the midline showed the hyperostosis to be composed of dense spongy bone (Fig. 2).

MICROSCOPIC EXAMINATION

The posterior lobe of the hypophysis appears normal. In the center of the flattened anterior lobe the epithelial cells have disappeared and have been replaced by dense connective tissue surrounding an irregular central cavity.

The tumor itself is composed of elongated spindle-shaped cells with oval vesicular nuclei. The cells are arranged in fascicles and whorls, some of which are calcified, and which are separated by a rather extensive connective tissue stroma.

A decalcified section along the midline of the entire sphenoid reveals the hyperostosis of the tuberculum sellae to be composed of moderately thick trabeculae of bone with masses of tumor cells occupying the large cancellous spaces (Fig. 3). The architecture of the tumor masses is similar to that seen in the primary tumor, although none of the cellular whorls is calcified. A layer of loose fibrous tissue separates the masses of tumor cells from the bony trabeculae in most of the spaces. In some areas definite rows of osteoblasts are present, an indication that the new bone has been laid down by endosteal stimulation and not by the tumor cells directly. The tumor invasion extends inferiorly to the epithelium of the underlying sphenoidal sinus. The remainder of the sphenoid is essentially normal. Sections of the optic nerves show that the nerve fibers have been compressed and destroyed, not by the hyperostosis but by direct extension of the primary tumor down the optic canals.

DISCUSSION

The idea, formerly held by some, that trauma plays a rôle in the production of hyperostoses (Spiller³ and Penfield⁴) associated with meningiomas seems disproved by recent reports of such involvement of structures at the base of the skull, presumably removed from the effects of any but the most severe blows to the head. Winkelman in 1930⁵ and Stender⁶ from this clinic have described cases of hyperostosis from meningiomas of the sphenoidal ridge. The present case demonstrates that another group of meningiomas of the base of the skull, the suprasellar meningiomas, may produce the same involvement.

The reasons are not clear for the apparent rarity of osseous involvement from meningeal tumors over the tuberculum. It is not unlikely, however, that tumors in this location, giving rise as they do

to serious loss of vision early in their course, cause the patient to seek surgical treatment earlier than do patients with meningeal tumors elsewhere. In the present instance the patient ignored the loss of vision which occurred for at least 15 years, during which time the tumor grew to tremendous size and had ample opportunity to invade the skull. It is also not impossible that small thickenings of the tuberculum may easily be overlooked, both in the ordinary X-ray views of the skull and at operation. In our case the only clear-cut clinical evidence for hyperostosis was in the oblique roentgenograms taken for visualization of the optic foramina. In the full lateral view the thickening of the tuberculum was not very evident.

The histological picture of the involved bone is similar to that of other meningiomatous hyperostoses. That the bony enlargement is a result of invasion of the cancellous spaces by tumor cells which stimulate osteoblasts to new bone formation has been demonstrated by Cushing⁷ and by Phemister.⁸ The latter has demonstrated further that a dense spongy arrangement of newly formed bone is characteristic of a slow rate of growth or a marked tendency to ossification. In the present case the trabeculae of bone were not as dense as in Phemister's cases, where the vault of the skull was the site of involvement. In connection with Phemister's statement that calcification in psammomas is in no way related to the bony thickening of the skull, it is interesting to note in our case that, while the primary tumor contained numerous calcified psammoma bodies, none was seen in the tumor masses in the hyperostosis of the tuberculum sellae.

SUMMARY

A case of meningioma producing a hyperostosis of the tuberculum sellae is described. The history consisted of gradual loss of vision of the left eye for 15 years and of the right for 4. Examination revealed complete blindness of the left eye and only light perception in the inferior nasal quadrant of the right visual field. Bilateral optic atrophy was present. Death from hemorrhage occurred during operation. Autopsy revealed a large, globular suprasellar meningioma which invaded the optic canals and produced a marked hyperostosis of the tuberculum sellae. The primary tumor contained numerous calcified psammoma bodies, while the tumor masses invading the bone and stimulating hyperostosis showed no calcification.

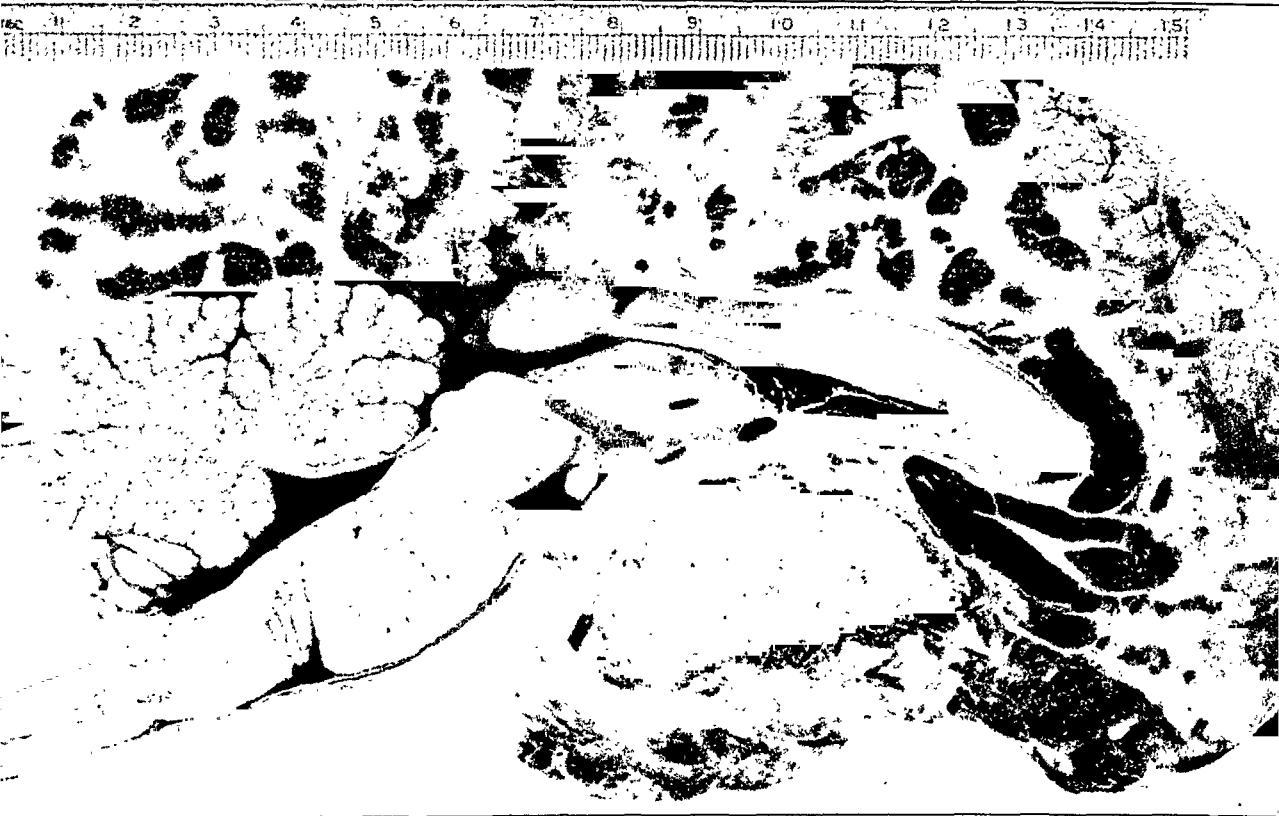
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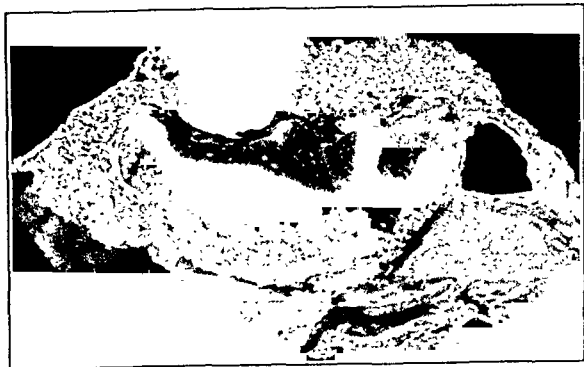
DESCRIPTION OF PLATE

PLATE 179

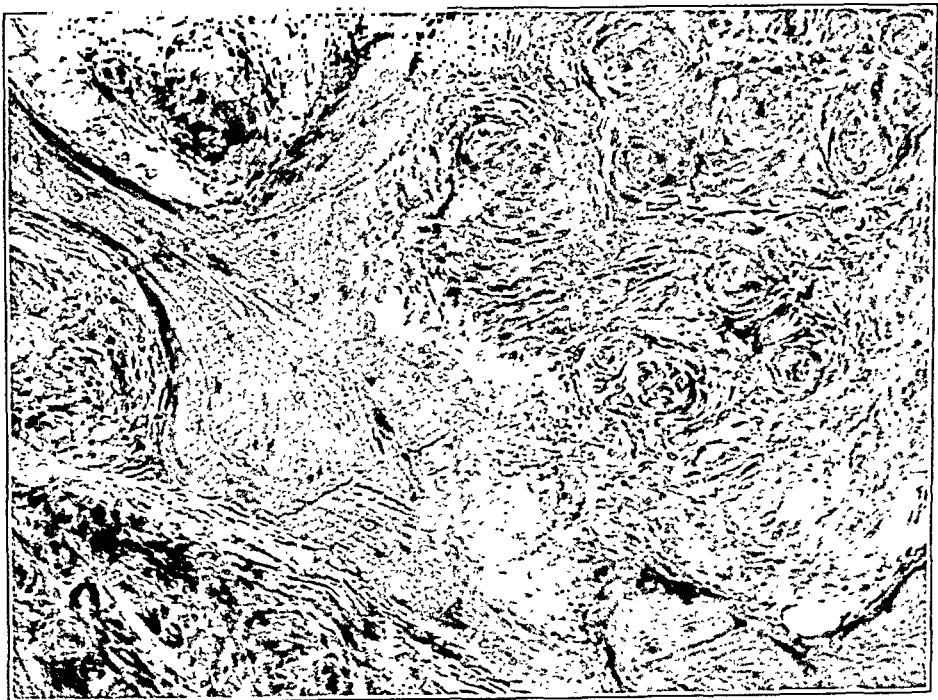
- FIG. 1. Sagittal section of the brain. The large meningioma lies beneath and anterior to the third ventricle. The hypothalamus, optic chiasm and neighboring structures which have been dislocated dorsally are seen resting on the upper surface of the tumor.
- FIG. 2. Sagittal section of the body of the sphenoid bone. The greatly thickened tuberculum sellae is seen just anterior to the sella turcica. It is obviously composed of spongy bone. Its entire thickness through to the dorsal wall of the sphenoidal sinus has been involved by tumor. Natural size.
- FIG. 3. Photomicrograph of the bone of the tuberculum sellae. Tumor tissue typical of a meningioma is seen filling the spaces between the bony trabeculae. The tumor is composed of the usual spindle-shaped cells arranged in bundles and whorls. Hematoxylin and eosin stain. $\times 125$.



I



2



3

PRIMARY INTRAMEDULLARY NEUROGENIC SARCOMA OF THE ULNA *

REPORT OF A CASE

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INTRODUCTION

The purpose of this paper is to record the occurrence of a tumor, primary in the ulna, having the characteristic structure of a perineurial fibroblastoma and presenting some histological evidence of malignancy.

The problems of histogenesis and classification of the tumors of nerves are still under dispute. This is especially true of the relation of the solitary tumors found on nerves to the generalized neurofibromatosis of von Recklinghausen. Penfield¹ in a recent publication has given an illuminating discussion of the question. To him the multiple fibrous nodules of von Recklinghausen's disease — the true neurofibromas — seem perhaps not neoplastic but rather of the nature of a reactionary fibrosis to some irritation, possibly neurotrophic, dependent upon congenitally faulty nerve insulation. Such nodules are tangled masses of collagenous tissue lacking any definite arrangement, and in their depths there are often to be found apparently intact nerve fibers.

Quite distinct from these knots of fibrous tissue is the truly neoplastic and usually solitary tumor mass with definite organoid structure found in connection with nerves. This type of tumor was originally called a neurinoma, or less exactly a neurofibroma. Subsequent study has given rise to two divergent concepts of its histogenesis. One group, chiefly the French investigators, believe the tumor to be derived from the cells of the sheath of Schwann, hence neurectodermal in origin, and call it the Schwannoma or peripheral glioma. The other group, whose view Penfield supports, claims the type cell to be the endoneurial fibroblast, and designates the tumor as a perineurial fibroblastoma. The most familiar example is perhaps

* Received for publication June 27, 1934.

the variously named intracranial tumor of the acoustic nerve, but localized tumors of the same type are commonly seen in the most varied places throughout the body. They are almost always slowly growing and well encapsulated tumors, though malignant change sometimes supervenes, especially in deep seated ones or following incomplete surgical removal.

Such growths are composed of parallel intertwining bundles of fairly mature fibroblasts, which show invariably a tendency to palisade arrangement of nuclei and often well marked undulant whorls. Between the cells is a moderately abundant quantity of fibrillary intercellular substance, very fine in texture and coloring only slightly with stains for collagen. Appropriate silver impregnation methods show that a considerable proportion of this intercellular substance is reticulum, in the form of long, straight, coarse fibrils, similar to the endoneurial reticulum described by Laidlaw.² Such reticulum stroma is a very constant feature, and together with the peculiar cell ordination is pathognomonic of the perineurial fibroblastoma. Fibroglia fibrils are often, but not always, demonstrable on the cells. Fat laden macrophages are frequently present, sometimes in great numbers. Areas of myxomatous degeneration with widely spaced stellate cells are often seen in the larger tumors.

Unlike the masses of von Recklinghausen's disease these tumors but rarely contain nerve fibers and then only at the periphery. Indeed their sole relation to generalized neurofibromatosis appears to be that occasionally within one of the multiple nodules a perineurial fibroblastoma may arise as a new and independent growth by a process of neoplastic release of included endoneurial fibroblasts.

REPORT OF CASE

The patient from whom the tumor to be described was obtained was a white painter, 55 years of age. He entered the hospital complaining of a swelling of the right forearm, which had been gradually increasing in size since first noticed a year previously. The swelling seemed to have caused no disturbance of motion or sensation, and only a mild aching pain in damp weather. About 6 months previous to noticing the tumor he had fallen, injuring his right arm, but not severely enough to prevent his working.

On examination he presented a large fusiform swelling 12 cm. in

greatest diameter in the middle third of the right forearm. The mass was hard, fixed to the ulna and not tender. The overlying skin was not warm or reddened. In spite of the size of the tumor, pronation and supination were free and complete. The record makes no mention of the presence of any of the stigmata of generalized neurofibromatosis.

X-ray plates (Fig. 1) showed the middle third of the ulna to be occupied by an expanding fusiform tumor arising within the medullary cavity and pushing a fairly complete thin shell of cortical bone before it. Within the mass, thin bony septa divided the tissue into large loculi, giving a marked "soap-bubble" appearance. No periosteal reaction triangle was present and there was no new bone formation in the tissue. X-ray diagnosis lay between benign giant cell tumor and some atypical form of fibrosarcoma.

Surgical exploration was decided upon. The ulna was exposed, the bony shell of the tumor broken through and the soft tumor tissue curetted out. The cavity left was cauterized with phenol and filled as well as possible with soft parts.

The tumor tissue removed was sent to the laboratory for frozen section diagnosis during the operation. A considerable quantity of rather soft, pinkish gray, friable tissue with small yellowish spots, larger translucent myxomatous areas and a few bony spicules was received. Frozen section showed a tumor consisting of loosely arranged spindle and stellate cells with very little delicate collagen between them. On the basis of rare mitoses and more frequent tumor giant cells a diagnosis of a slowly growing sarcoma was made, and because of the whorls, seen imperfectly in the unfixed section, it was suggested that the tumor might be of neurogenic origin.

Three days following operation celloidin sections were available. As in the frozen section, occasional mitoses and fairly numerous tumor giant cells were present. The whorls and palisades were now quite plainly evident and, accordingly, a diagnosis of a slowly growing, well differentiated neurogenic sarcoma was made.

Because of the highly malignant character of the cases of neurogenic sarcoma reported by Geschickter,³ and others, surgical consultants were almost unanimous in advising amputation. After X-ray plates of the chest and pelvis had shown no evidence of metastases, the arm was amputated 6 cm. above the elbow. The large nerves at the site of amputation were found grossly normal in

appearance. The patient made an uneventful recovery and was discharged 2 weeks after operation. At the time of writing, 20 months after operation, he has shown no signs of recurrence or metastasis.

The ulna obtained from the amputated arm showed on the medial aspect a deep, crater-like defect 10 by 4 cm., filled with the flexor muscles. Between muscle and bone was some hemorrhage, and next the bone a narrow incomplete zone of grayish tissue. In microscopic section this appeared to consist only of granulation tissue and a small amount of newly proliferating bone. No recognizable tumor tissue was present. The preservation of free rotatory movement, in spite of the size of the tumor, was apparently due to the fact that the tumor expanded the cortex of the ulna only on the medial side and hence did not interfere with the motion of the radius.

MATERIAL AND TECHNIQUE

The following description is based on the material removed by curettage at the first operation. The tissue was fixed in Zenker's fluid and in 10 per cent formalin. Paraffin sections were stained with phloxine-methylene blue, phosphotungstic acid hematoxylin, hematoxylin and eosin, Weigert's elastic tissue stain, Mallory's aniline blue connective tissue stain and Masson's trichrome modification of it, and del Rio-Hortega's silver carbonate for reticulum, as modified by Foot for Zenker-fixed paraffin sections. Frozen sections of formalin-fixed tissue were stained with scharlach R and by the Smith-Dietrich method for lipoids. The blocks from which they were cut were mordanted, embedded in celloidin, and sections stained by the classical method of Weigert for myelin. Frozen sections were also impregnated by the Gros-Schultze and Bielschowsky methods for nerve fibers, and by a method devised by Penfield⁴ for oligodendroglia and Schwann cells.

DESCRIPTION OF TUMOR

Microscopically the tumor consists of a loose but cellular tissue made up of spindle and stellate cells. In the greater portion they are arranged in the form of random bundles, but scattered here and there are numerous, loose, concentric whorls of large size, some in the form of longer undulant structures in which there is a tendency to palisading of nuclei (Fig. 2). The cells are nowhere closely packed

but are separated by a moderate amount of very delicate, fibrillary intercellular substance. Occasional areas are edematous, and both cells and fibrils are few and widely separated but preserve the suggestion of whorls (Fig. 3). No coagulum is present in the spaces between them. There are also numerous large areas in which the tissue is closely packed with great numbers of phagocytes having a rather uniform, foamy cytoplasm (Fig. 4). The vessels are sparse and are for the most part small, well formed arterioles and capillaries. In a few sections there are coarse strands of dense, collagenous connective tissue which probably represent parts of the fibrous periosteal capsule.

On closer examination the cells are seen to be of fairly large size and spindle, stellate or sometimes round in shape. The cytoplasm is moderately abundant, dense, homogeneous and basophilic, and in the round cells sometimes granular or vacuolated. The nuclei are vesicular with a medium amount of evenly distributed chromatin and one, occasionally two or more, large nucleoli. Multinucleated forms are exceedingly numerous, constituting the majority of cells in many fields. Here and there they have the shape of multinucleated ribbons of cytoplasm, somewhat resembling the proliferating neurilemma in the regenerating end of a cut nerve (Fig. 5). There is, however, no suggestion of a collagenous sheath about them. Some cells appear to have processes surrounding and forming a partial sheath for masses of material resembling segments of myelin sheaths. Many others contain round or oval masses, or vacuoles of similar appearing material.

With phosphotungstic acid hematoxylin the delicate, intercellular fibrillary material is colored pale reddish brown, or is almost unstained. Fibroglia fibrils are well shown in the coarse, adult connective tissue fragments of the capsule, and in the adventitia of the larger vessels. The tumor cells have rarely one or more long, delicate fibrils coursing over the cell body (Fig. 6). These fibrils are found on the spindle cells and rarely on the stellate forms, but not on the swollen, multinucleated giant cells. More commonly, perhaps, the tails of the spindle cells stain heavily in the manner of spongioblasts. Occasionally a small group of centrosomes is visible near the nuclei in the large multinucleated polygonal cells.

Turning from the cells to a consideration of the intercellular substances, as displayed by suitable technique, the aniline blue con-

nective tissue stain reveals a large amount of finely divided collagen, and the more precise and powerful trichrome modification of Masson shows even more, between the cells and crosswise in the whorls. The abundance and character of this material is demonstrated fully, however, only by a silver impregnation such as the Hortega-Foot method. Sections so prepared show a great quantity of long, straight or wavy, wire-like fibrils running between the cell bundles and circularly and crosswise in the whorls (Fig. 7). A large amount of the intercellular material in this tumor is silver-positive, in other words it is reticulum. Only the coarser masses, some doubtless remains of preëxisting tissue, stain as collagen in the metallic impregnation.

The tumor cells apparently produce no elastic tissue. Rarely short fragments of elastic fibrils may be found among the tumor cells, and the few dense masses of adult connective tissue already mentioned contain fairly numerous, large elastic fibers. All these, however, appear most likely to be derived from preëxisting tissue, possibly the periosteum.

The large accumulations of fat-laden phagocytes are a curiously frequent, though not pathognomonic, feature of the perineurial fibroblastoma connected with both the acoustic and peripheral nerves. They are a very prominent item in this tumor and no entirely satisfactory explanation can be given for their presence. Where few in number they usually cluster about the adventitia of small vessels. In other places they fill whole low power microscopic fields, yet no evidence of degeneration is visible in the adjacent tumor tissue. They are far too numerous to have resulted from the destruction of included myelinated nerve fibers, as Wlassics⁵ suggested. It is possible that the lipoid material is derived from the fat of the marrow replaced by the tumor. Against this origin, however, is the fact that fatty macrophages, while commonly seen in inflammatory lesions, are but rarely present in any of the varieties of bone tumors.

If one accept the Schwannian origin of the perineurial tumors, the attractive explanation suggests itself that the tumor cells, like normal Schwann cells, are producing myelin and discharging it free into the tissue where it is picked up by phagocytic cells. The phagocytes stain brilliantly with scharlach R, and occasional tumor cells, both in their neighborhood and at a distance, contain droplets staining as fat. However, the Smith-Dietrich reaction for lipoids is negative, save for a few granules in rare tumor cells, and the Weigert

method for myelin reveals no trace of myelinated nerve fibers or free myelin in the tissue. In unstained frozen sections examined by polarized light much of the fatty material in the phagocytes is doubly refractile.

No necrosis is seen in the tumor. The vessels are few in number, small and well formed, and do not show the hyaline degeneration often described in these tumors. Many of the vessels have a moderate perivascular lymphocytic infiltration. A few small hemorrhages, probably the result of trauma incident to curettage, are scattered in the tissue.

DISCUSSION

This tumor is classified with the perineurial fibroblastomas of the acoustic and peripheral nerves because, like them, it presents a combination of spindle cell and myxomatous tissue in which the cells are often arranged in whorls and palisades, and have between them a delicate fibrillary stroma consisting in large part of reticulum. Probably the strongest evidence of relationship is the presence of striking numbers of whorls and palisades differing only in looseness of texture from those of the generally recognised perineurial fibroblastomas.

The reticulum stroma is also a very characteristic feature, as Penfield has pointed out, and is uniformly present in all of our specimens of perineurial tumors. The work of Mallory and Parker⁶ has demonstrated that reticulum is the same chemical substance as collagen in merely a finer state of subdivision, yet it is certainly significant that the stroma of all perineurial tumors should be composed quite largely of this finely divided form of collagen. Reticulum in small amount is present in the stroma of most tumors, but according to the investigation of Foot and Day,⁷ it forms the predominant stroma only in rapidly growing tumors. The perineurial fibroblastomas, however, including the case here reported, are slowly growing, so that the constant presence of large quantities of reticulum is a diagnostic feature of considerable importance and suggests an inherited manner of cellular growth maintained even in neoplasms.

The intramedullary position of this tumor is well shown in the accompanying X-ray plate (Fig. 1). This intramedullary position of a perineurial fibroblastoma is, as far as I can ascertain, unique. The nearest resemblance seems to be a case reported by Brooks and Lehman,⁸ in which a periosteal cyst of the tibia formed one of multi-

ple bony deformities associated with generalized neurofibromatosis. The tissue removed is described simply as "typical neurofibroma as in the skin," but no details or illustrations are given.

A considerable number of tumors designated as neurogenic sarcoma are reported in the literature, but all seem to have been primary in the soft parts and to have involved bone only secondarily. Judging from the published illustrations they were all rapidly growing and highly malignant tumors, so anaplastic that the neurogenic origin of many is by no means clear.

The tumor in our case no doubt took origin from one of the sparse nerve trunks within the marrow cavity of the ulna. Nerve fibers in bone, usually accompanying vessels, are described in most textbooks of histology, and rarely in aplastic marrows small groups of myelinated fibers may be seen.

The classification of this tumor as a slowly growing sarcoma depends, perhaps rather empirically, as with other mesodermal tumors, upon the preponderance of cells over intercellular substance and upon the occurrence of occasional mitotic figures. The malignancy is certainly of a low grade and with such complete surgical removal recurrence or metastasis seems unlikely to occur.

A discussion of the vexed problem of Schwann cell versus fibroblastic origin of this tumor has been purposely avoided from a belief that to be of value such discussion would require a wider base than that afforded by a single case. Because of the opportunity offered by the many clearly visible tumor cells a trial was made of a modification of Hortega's method for oligodendroglia devised by Penfield,⁴ and said to stain also the Schwann cell selectively. No appearance of specific staining of the tumor cells could be produced.

SUMMARY AND CONCLUSIONS

A case of a solitary intramedullary tumor of the ulna presenting the histological structure of a perineurial fibroblastoma and some evidence of sarcomatous growth has been described in detail.

The occurrence of tumors of the perineurial type primarily in bone must be exceedingly rare, this apparently being the first case recorded. Estimate of their biological character is accordingly uncertain, but on histological evidence they seem, unlike the neurogenic sarcoma of soft tissues, to be a tumor of low grade malignancy.

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DESCRIPTION OF PLATES

PLATE 180

FIG. 1. Anteroposterior plate of the right forearm showing the tumor in the ulna.

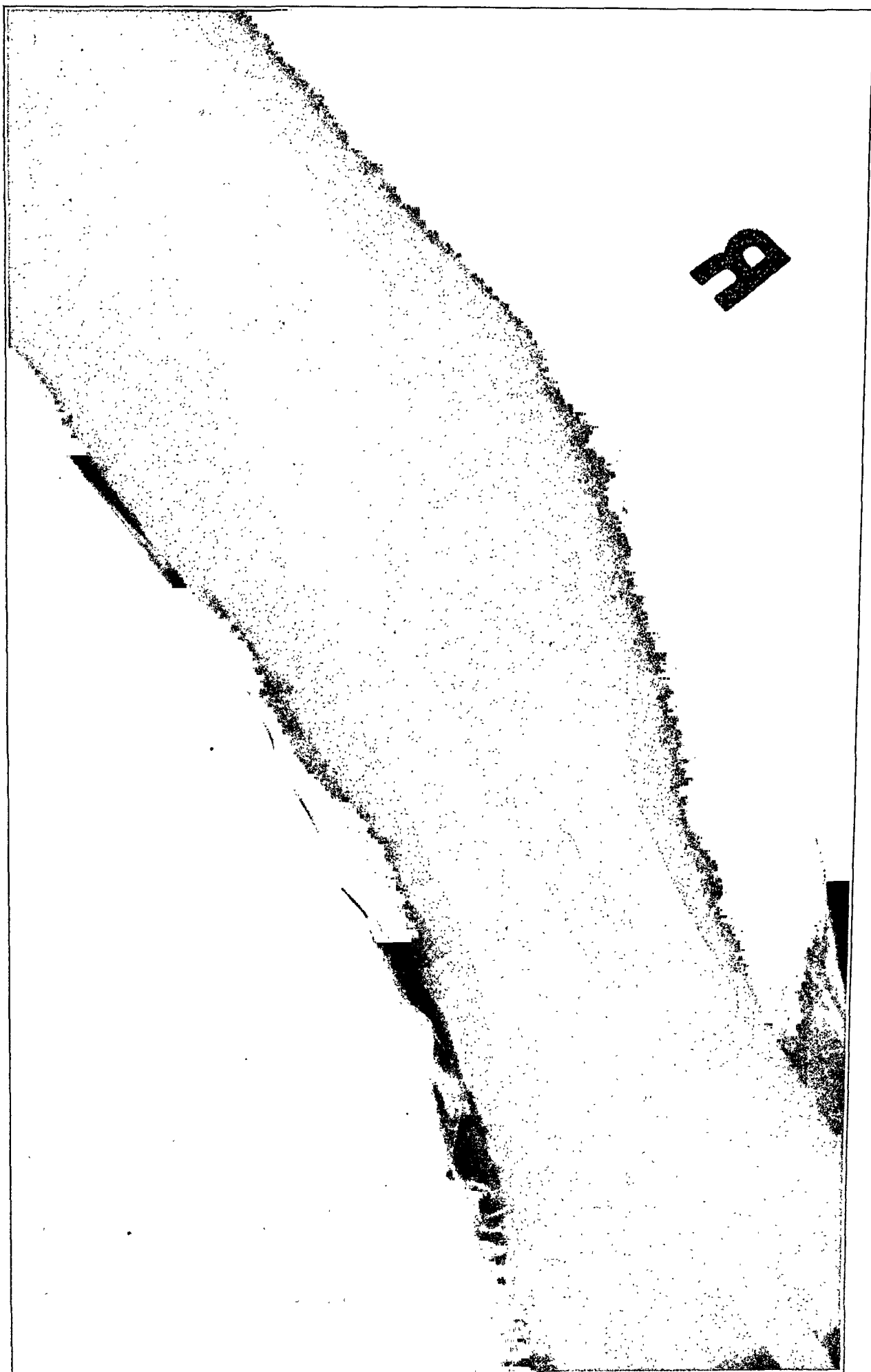
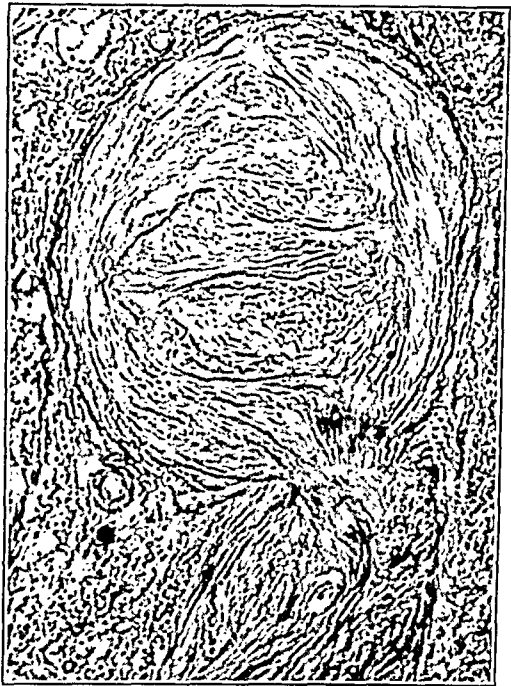


PLATE 181

- FIG. 2. Cellular portion of the tumor, showing whorls and a loose palisade.
- FIG. 3. Area of myxomatous type of tissue.
- FIG. 4. Field showing extensive accumulation of fat-laden phagocytes. Lymphocytic infiltration about a small arteriole.
- FIG. 5. Multinucleated tumor giant cell having the form of a ribbon of cytoplasm.
- FIG. 6. A fibroglia fibril on the surface of a tumor cell. Phosphotungstic acid hematoxylin stain.
- FIG. 7. Hortega-Foot impregnation showing the abundance and complex arrangement of reticulum in a whorl.



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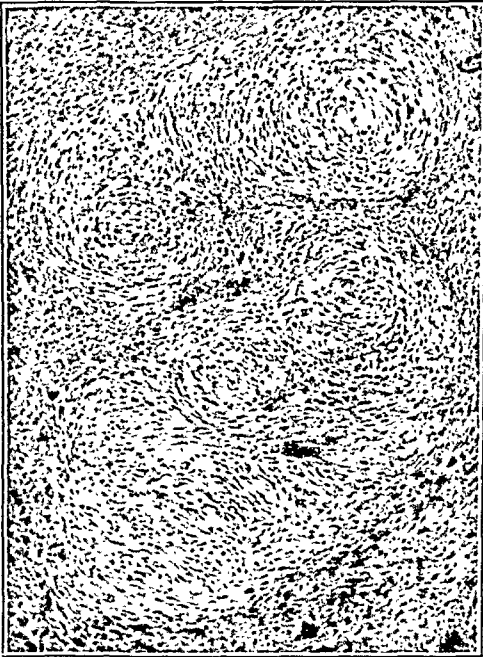
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THE RELATION OF INCREASED INTRA-ABDOMINAL PRESSURE TO THE LIVER LESIONS OF ECLAMPSIA *

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Attention has been directed since 1886 to the occurrence of hepatic lesions in eclampsia. In that year Jürgens¹ and in 1888 Klebs² noted the occurrence of hemorrhagic hepatitis. In 1890 Pilliet³ described the microscopic appearance of the liver lesions in 12 cases of eclampsia. Schmorl,⁴ in a monograph based upon the autopsies of 17 women dead of eclampsia, stated that he had found in every case lesions of the liver which he believed were so characteristic that their presence justified the diagnosis of eclampsia without further knowledge of the history of the case. Microscopically these lesions consisted of areas of necrosis, with or without hemorrhage, located in the neighborhood of the smaller portal vessels, that is, a peripheral necrosis rather than the central necrosis commonly found in a variety of conditions. Many investigators confirmed these observations within the next few years: for example Bouffe de Saint Blaise⁵ demonstrated such lesions in the livers of 42 consecutive cases and in 1902 Schmorl⁶ reported finding them in 71 of 73 autopsied cases of eclampsia, the remaining 2 cases showing fresh complete thrombosis of the portal vein. Williams⁷ recently stated that he had been able to demonstrate similar lesions in all the eclamptic livers that he had examined and agreed with Schmorl that such lesions occurred in no other condition. So characteristic are these lesions that many observers believe they represent the primary lesion of eclampsia, the manifestations of which are considered due to an impairment of hepatic function, "hepatotoxemia." There are other physicians,^{8, 9} however, who report that the livers of eclamptic patients may occasionally show very little abnormality. Theobald¹⁰ states that typical eclamptic liver lesions may be found rarely in patients dying of

* Received for publication June 29, 1934.

general peritonitis and in those with pneumonia. Mallory,¹¹ many years ago, suggested that the appearance of hemorrhages in the liver in eclampsia could probably best be explained as the result of the purely mechanical forces involved in the convulsions. He noted the occurrence of similar lesions in the liver of a man dying from meningitis. Quite recently Theobald¹⁰ has revived the mechanical theory for the etiology of eclamptic liver lesions. He has performed a number of interesting experiments on animals in which he believes that he has produced the eclamptic type of liver lesion by repeatedly raising the intra-abdominal pressure. If it can be shown that liver injury is a secondary phenomenon, the theory that eclampsia is a primary hepatotoxemia must be abandoned. Theobald's observations are, therefore, of such importance that it was considered advisable to repeat them. The technique used by Theobald¹⁰ in his experiments was as follows.

"The dog was given an injection of morphine sulphate gr. i and half an hour later anaesthesia was induced with a mixture of equal parts of chloroform and ether. The animal was then tied on its back and a litre (sometimes a little more) of sterile normal saline solution was introduced with all antiseptic precautions into the peritoneal cavity through a trocar inserted in the middle line. The reason for the introduction of the saline was because without it, it was found impossible to raise the intraperitoneal pressure much above 40 cm. of saline solution. The fluid having been run in, two dusters were placed round the abdomen and drawn tight, by which manoeuvre the intraperitoneal pressure was raised to between 80 and 100 cm. of saline solution. This manoeuvre will, for the sake of convenience, be referred to as a 'fit,' each of which lasted two minutes unless the contrary is stated."

His first animal was sacrificed 3 hours after the experiment in which the intra-abdominal pressure had been raised 9 times. The liver is described as follows: "The cells are swollen and granular, the nuclei stain badly and the sinusoids are widely dilated. The feature of interest, however, is the fact that Glisson's capsule has been most severely affected and in nearly every area the cells are swollen and the structures, including the bile ducts, are seen to be disintegrated." These changes cannot be made out clearly in the halftone reproduction (Fig. 1¹⁰) of the photomicrograph. A second animal received only morphine. The number of "fits" and the time of sacrificing

are not stated. No changes are apparent in the reproduction (Fig. 2¹⁰) of the photomicrograph, although it is stated that changes similar to those quoted above are present. A third animal, receiving as an anesthetic the chloroform-ether mixture and having its intra-abdominal pressure raised 5 times one day and 6 times the next day, was sacrificed 24 hours later. The liver showed "extensive degeneration and necrosis, chiefly central but extending in not a few areas to the periphery of the lobules." These changes are illustrated in the photomicrograph (Fig. 3¹⁰), and do not appear at all characteristic of the eclamptic type of lesion in woman. Another animal killed 14 days after having had its intra-abdominal pressure elevated 3 times in one day and 5 times on the next day, showed extensive necrosis, the location of which is not specified in the text and cannot be determined from the photomicrograph (Fig. 4¹⁰). A pregnant cat, killed 18 hours after the intra-abdominal pressure had been raised, showed extensive degeneration in the liver "with no particular lobular distribution." A dog, whose intra-abdominal pressure had been raised 30 times at 3 minute intervals, each time for 30 seconds, was killed 24 hours later. "Sections (of the liver) show large areas of necrosis, chiefly central, and a few areas of hemorrhagic necrosis situated in the periphery of the lobules."

EXPERIMENTAL

Our procedure was identical with that of Theobald, except for the fact that morphine was not employed in any instance and ether alone was used as an anesthetic in 7 dogs. A mixture of equal parts of chloroform and ether, as employed by Theobald, was used in 4 other dogs. The protocols of the 11 experiments follow.

Dog No. 29: Ether anesthesia. Intra-abdominal pressure raised 18 times, immediately following which respirations ceased, 72 minutes after the first elevation of pressure. Autopsy revealed edema of the lungs and an elevated diaphragm. The liver was grossly and microscopically normal.

Dog No. 25: Ether anesthesia. Intra-abdominal pressure raised 10 times, immediately following which respirations ceased, 40 minutes after the first elevation of pressure. The liver was grossly and microscopically normal.

Dogs Nos. 23, 24 and 28: Ether anesthesia. Intra-abdominal pressure raised 20 times during a period of 80 minutes in each animal. Each dog was sacrificed 72 hours after the completion of the experiment. All three livers were grossly and microscopically normal.

Dog No. 26: Ether anesthesia. Intra-abdominal pressure raised 15 times during a period of 60 minutes on 1 day and this repeated 2 days later. The

animal was sacrificed 72 hours after the 2nd day on which the pressure was raised. The liver was grossly and microscopically normal.

Dog No. 27: Ether anesthesia. Intra-abdominal pressure raised 10 times within 40 minutes on 1 day, and 5 times within 20 minutes 5 days later. The animal was sacrificed 5 days after the 2nd day on which the pressure was raised. The liver was grossly and microscopically normal.

Dog No. 20: Anesthesia maintained with equal parts of chloroform and ether. Intra-abdominal pressure raised 10 times. The animal was sacrificed 72 hours later. The liver showed widespread central necrosis, occasionally extending to the periphery of the lobule.

Dog No. 30: Anesthesia maintained with equal parts of chloroform and ether. Intra-abdominal pressure raised 10 times within a period of 40 minutes on 1 day, and this repeated 72 hours later. The animal was sacrificed 72 hours after the 2nd day on which the pressure was raised. The liver showed extensive central necrosis, occasionally extending to the periphery of the lobule.

The next two animals (Nos. 21 and 22) were merely anesthetized with equal parts of chloroform and ether.

Dog No. 21: The anesthesia was maintained for 1½ hours. This animal received more of the anesthetic mixture than either of the above dogs, Nos. 20 and 30. It was sacrificed 72 hours later. The liver was grossly and microscopically normal.

Dog No. 22: The anesthesia was maintained for 1½ hours. This dog received much less of the anesthetic mixture than dogs Nos. 20, 30 and 21. It was sacrificed 72 hours later. The liver showed most extensive central necrosis with only a border of cells remaining intact about the periphery of the lobules.

DISCUSSION

The above observations on dogs Nos. 20 and 30 confirm those of Theobald, inasmuch as extensive necrosis of the liver, chiefly central, but occasionally extending to the periphery of the lobule, occurred when the intra-abdominal pressure was raised in animals which had received chloroform-ether anesthesia. However, 1 of 2 animals receiving merely this same anesthetic, developed even more extensive liver lesions. It is likewise to be emphasized that 7 animals anesthetized with ether alone, 5 of which survived 3 or more days, although subjected in most instances to even more numerous elevations of the intra-abdominal pressure than Theobald's animals, failed to show any lesions of the liver. The significance of the use of chloroform as an anesthetic is apparent. It is so well known that chloroform can produce extensive central necrosis of the liver that this need not be enlarged upon here. It is also apparent that the lesions produced by Theobald¹⁰ are entirely consistent with chloroform necrosis and are not at all characteristic of the periportal lesion of eclampsia.

SUMMARY AND CONCLUSIONS

1. Elevation of the intra-abdominal pressure, according to the technique of Theobald, was without effect upon the livers of 7 dogs anesthetized with ether.

2. A chloroform-ether anesthetic mixture, such as that employed by Theobald, resulted in extensive central necrosis of the liver in 3 of 4 dogs, only 2 of which were subjected to increases of intra-abdominal pressure.

3. The theory that the liver lesions of eclampsia are due to increased intra-abdominal pressure, although an attractive hypothesis, must be considered as yet unproved.

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CHANGES PRODUCED IN THE CENTRAL NERVOUS SYSTEM OF THE MOUSE BY THE VIRUS OF ST. LOUIS ENCEPHALITIS *

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Transmission of the virus of St. Louis encephalitis to mice has been reported by two groups of workers: (1) Muckenfuss, Armstrong and McCordock,¹ and (2) Webster and Fite.² The latter workers used a special strain of mice peculiarly susceptible to neurotropic virus, and they also found that Swiss mice would contract the disease, but that the stock strain of mice used in the Rockefeller Institute was affected in a "relatively small percentage of animals." Muckenfuss and associates demonstrated that stock strains of mice available in St. Louis were uniformly susceptible. Both of the above groups of authors have briefly described the symptoms and lesions produced in mice by the virus.

MATERIAL

Four strains of virus were used: three strains isolated in this laboratory, namely, the Freeman, Barnes, and Daily strains, and one obtained by Webster designated as No. 3. Two hundredths of a cubic centimeter of 10 per cent brain emulsion was inoculated into the brains of white mice of the Swiss strain and white mice obtained from three breeders in the St. Louis area.

Several hundred brains were removed with the least possible traumatization and fixed in Zenker-acetic solution. Paraffin sections of blocks taken from various levels were stained with hematoxylin and eosin. For particular studies animals were killed at definite intervals after inoculation, or when moribund. Special fixatives and staining methods were used to demonstrate particular changes.

COURSE OF ILLNESS

The mice usually remain well until the 5th day after inoculation, but occasionally they show signs of illness and die on the 4th day, when highly virulent material has been used. The first signs are a ruffling of the fur and a hunched posture. At this stage an exaggerated response is obtained on stimulation. Then the mouse may assume the "encephalitic position,"³ sitting erect with hind legs

* Received for publication June 24, 1934.

spread apart and the tail used as a support. Tremors of extremities, head and tail are usually observed. Convulsions and paralyses, both spastic and flaccid, are common and occasionally the mice run in circles. Some animals apparently have disturbance of vision for they run into objects and fall off the edge of the table.

The disease may progress very rapidly and kill the mouse in a few hours or it may be protracted for several days. In the latter case the paralyses frequently become generalized and the respirations slow and shallow.

PATHOLOGY

Webster and Fite² have described the lesions produced in mice by the St. Louis encephalitis virus as: "accumulations of mononuclear cells in the pia, about the blood vessels in the brain and spinal cord and in scattered foci. The pyramidal cells of the cornu ammonis and lobus pyriformis are injured, thus disturbing the regular architecture of these regions. Certain nerve cells of the basal ganglia and anterior horn cells of the spinal cord appear damaged and collared by mononuclear cells."

Little need be said about the gross appearance of the brain. The surface vessels are usually injected and the brain frequently has a diffuse pinkish appearance. The site of inoculation in the left area striatum⁴ at the left of the thalamus is generally apparent as a small red depression.

Microscopically the most striking and constant lesion is the collection of cells about vessels. This is observed in all parts of the brain and in the spinal cord. There are three types of cellular reaction, all of which may be exhibited in the same brain.

The first type consists of a collection of mononuclear cells in the Virchow-Robin space. The predominant cell in the perivascular cuff is the lymphocyte, but not infrequently there are also mononuclear wandering cells, some with elongated oval nuclei and scanty cytoplasm, others with kidney-shaped nuclei and abundant cytoplasm. The width of the cuff varies from one layer of cells to four or five. There is no cellular infiltration in the brain substance immediately surrounding the vessel (Fig. 1).

The second type of reaction is characterized by the presence of cells in the brain substance adjacent to vessels, but with none of the previously described cells in the Virchow-Robin space. With hema-

toxylin and eosin stain the cells consist of irregularly shaped nuclei and little or no cytoplasm. Some of the nuclei are elongated and rod-like with slightly blunted ends, others are club-shaped, and some are curved and twisted on themselves (Fig. 2). In material fixed in formol-ammonium bromide and stained by del Rio Hortega's method, many of these cells show the typical appearance of microglia (Fig. 4).

The third type is a combination of the first two. Here there is a perivascular cuff and also a proliferation of microglia in the brain substance about the vessel (Fig. 3).

All three reactions are usually present in the same mouse. Probably the most frequent type of involvement is that in which there is both a perivascular cuff and a collection of glia cells near the vessel. Cuffing alone is more common than the simple glia proliferation.

Mice vary individually, not only in the severity of reaction to the same dose of virus, but also to some extent in the type of cellular collections about vessels; thus in a particular lot some mice will show cuffing predominantly with only a slight glial reaction, while others display a glial response equal to or greater than the perivascular.

Different strains of white mice apparently show a slight variation in response to the virus. Swiss mice in general have more clearly defined and thicker perivascular cuffs. Mice obtained from three different stocks in the St. Louis area, among which there was probably more or less cross-breeding, seem, in general, to show smaller and less clearly defined cuffs with a somewhat greater proliferation of glia.

The reaction about vessels appears as early as the second day after inoculation. At this time it is not intense, although cuffs one to two cells thick and some microglia proliferation in the surrounding brain tissue are observed in the Swiss mice. The region of inoculation is first affected. By the 3rd day the lesions are scattered diffusely throughout the brain with less involvement of the cerebellum than of the cerebrum.

Degeneration of ganglion cells is another constant lesion. Various changes are observed, such as swelling, chromatolysis, and pyknosis of the nucleus, disappearance of Nissl granules with a homogeneously purple-staining of the cytoplasm, and finally such complete destruction of the cell that it stains a deep pink. The 2nd day after inoculation there is some degeneration of ganglion cells in an area

limited almost entirely to the region of inoculation. By the 3rd day the degeneration is apparent throughout the brain in a patchy distribution. Small areas of ganglion cells in Ammon's horn show the change frequently (Fig. 5). The patches of degeneration are often observed in the vicinity of a vessel. Most of the ganglion cells within the circumference of the glial reaction show changes, but the process rapidly diminishes beyond it.

In mice killed when moribund the widespread severe destruction of nerve cells is striking. No area is immune, but there are still islands of normal cells. The cortical substance in the occipital and striate areas, and in the parietal region overlying Ammon's horn, is more severely affected than are the basal nuclei and the brain stem. Neuronophagia is observed. Some of the Purkinje cells of the cerebellum take a deep purple stain with hematoxylin and eosin but rarely show the complete necrosis seen in cells in the cerebrum. The degeneration is more marked in mice inoculated with a heavy dose of the virus.

A third constant feature of the disease is an infiltration of the meninges with cells (Fig. 6). These cells are sometimes grouped about vessels but are usually scattered in the subarachnoid space. Lymphocytes predominate but there are also monocytes and an occasional polymorphonuclear leukocyte and plasma cell. The meningeal reaction varies in intensity and may be fairly marked as early as 48 hours after inoculation.

Focal collections of cells occur in brain tissue of mice less frequently than in human or monkey material. Occasionally, small collections of glia cells or of mononuclears apparently not related to a vessel are seen (Fig. 7). In order to determine more definitely the relation between these collections and vessels, serial sections of two mouse brains were made. All of the focal collections in these two brains appear to be a part of a cuff or a glial reaction about a vessel. The blood vessels and small capillaries are all congested, frequently to a marked degree. Hemorrhages into the perivascular space or into the brain substance are occasionally seen. No evidence of demyelination is present in sections stained by Loyez' method. No intranuclear inclusion bodies are found in Giemsa preparations, nor are the cytoplasmic inclusions described by Nicolau and co-workers^{5, 6} in normal mice obtained in Paris and England observed in the four strains of mice at our disposal.

The spinal cord shows well developed lesions. The three types of cellular response observed about vessels in the brain are frequently seen in the cord, but here the reaction is generally less severe. Degeneration of nerve cells, mainly the motor cells of the anterior horn, occurs (Fig. 8). Swelling of the motor cells appears as early as the 2nd day, before there is involvement about vessels. By the 5th day degeneration of the ganglion cells is marked. The cervical and upper thoracic regions of the cord are most frequently involved.

Sections of other organs were made in a number of instances. No notable changes are observed, except for the occasional presence of fat droplets in liver cells.

DISCUSSION

Spontaneous encephalitis occurring in mice which appeared healthy during life has been described by Cowdry and Nicholson.⁷ The protozoan-like parasites and the lesions in the brain were similar to those described in rabbits by several investigators.^{8, 9, 10} Levaditi¹¹ has named the parasites "*microsporidia*," and further designated them as "*Encephalitozoön cuniculi*."

The lesions of spontaneous encephalitis in mice were described by Cowdry as follows: "Focal infiltrations are most frequently met with and perivascular, meningeal and subependymal infiltrations were seldom seen in their absence. It was . . . a common experience to find focal infiltrations when the other varieties were difficult to detect. . . . In many cases . . . it was possible to trace complete continuity between a meningeal infiltration on the surface, its extension into the brain substance as a perivascular infiltration about a penetrating blood vessel, and its termination as a focal lesion either at the end of a vessel or to one side of it. . . . The same kinds of cell were found to participate in the infiltrations quite irrespective of their location (meningeal, perivascular, focal, or subependymal). In all . . . specimens lymphocytes predominated, but a few plasma cells and macrophages were also noted. The latter were particularly numerous at the centers of the focal lesions in the presence of slight necrosis and in the absence of lymphocytes, which were clumped about the periphery."

We have not encountered evidence of spontaneous encephalitis in mice of the Swiss stock. Occasionally this condition was observed in mice of the St. Louis stocks. In one lot of a hundred mice, which

appeared in poor general condition, the brains of eight were sectioned as controls. Four had the lesions described by Cowdry and in three of these microsporidia were found. This is an unusually large proportion and in other apparently healthy lots from the same breeder evidence of spontaneous encephalitis was infrequent.

In general, the spontaneous type of encephalitis which we have encountered has corresponded with the description given by Cowdry. In one instance we found the parasites scattered among the cells infiltrating the meninges. In our mice we have sometimes seen glia cells in the focal reactions in addition to the mononuclear leukocytes and lymphocytes.

Cowdry described lesions in the cervical cord and stated that they probably extend farther down the cord. We have examined spinal cords from three mice in which the brain contained parasites and lesions. In each of these a mononuclear infiltration has been found in the spinal meninges as well as around vessels, and also collections of mononuclear and glia cells about a focus of necrosis. Ganglion cell degeneration has not been observed in these sections. Microsporidia were not demonstrated in the cord. The lesions were observed in the cervical, thoracic and lumbar regions.

Neither in the previous description of spontaneous encephalitis nor in our experience was a glial reaction seen about the vessels with cuffs, except when the cuffed vessel was near a focal reaction which contained glia cells.

The desirability of using a strain of mice free from epizootic encephalitis when working with a neurotropic virus cannot be over-emphasized. Even when the lesions are severe it is difficult to distinguish the spontaneous from the experimental type of encephalitis, but there are several criteria which help to differentiate the two. The presence of microsporidia is of course positive evidence of the epizootic origin of the disease. Proliferation of microglia about the perivascular cuffs is rarely seen in spontaneous encephalitis, whereas it is a constant finding in the experimental type. Degeneration of ganglion cells of the brain and spinal cord is much less frequent and less severe in the former. On the other hand, the focal reactions are more numerous, more marked and of a slightly different character in the former. Of less importance is the tendency in the epizootic disease for the infiltrations to extend from the meninges into the brain substance along vessels and in sheets. This is not so apparent

in experimental encephalitis, where cuffing is rather evenly distributed throughout the brain.

The pathological picture produced by the St. Louis encephalitis virus is comparable in humans, monkeys and mice. There is no significant difference in the lesions found in man and monkey. The process in mice seems more severe than in the other two species; in general the cuffing, ganglion cell degeneration and meningeal reaction are more marked. The glial reaction about vessels, which is common in mice, is rarely, if ever, seen in man and monkey, whereas the glial nodules which are common in the two latter are relatively infrequent in mice.

SUMMARY

1. A description of the pathological lesions produced in the mouse by the St. Louis encephalitis virus is recorded.
2. A comparison is made between the lesions in the mouse and those in man and monkey.
3. A differentiation between the lesions in mice in experimental encephalitis and in epizootic encephalitis associated with microsporidia is discussed.

NOTE: We wish to express our indebtedness to Dr. Howard A. McCordock for his helpful criticism and for his aid in preparing the photomicrographs.

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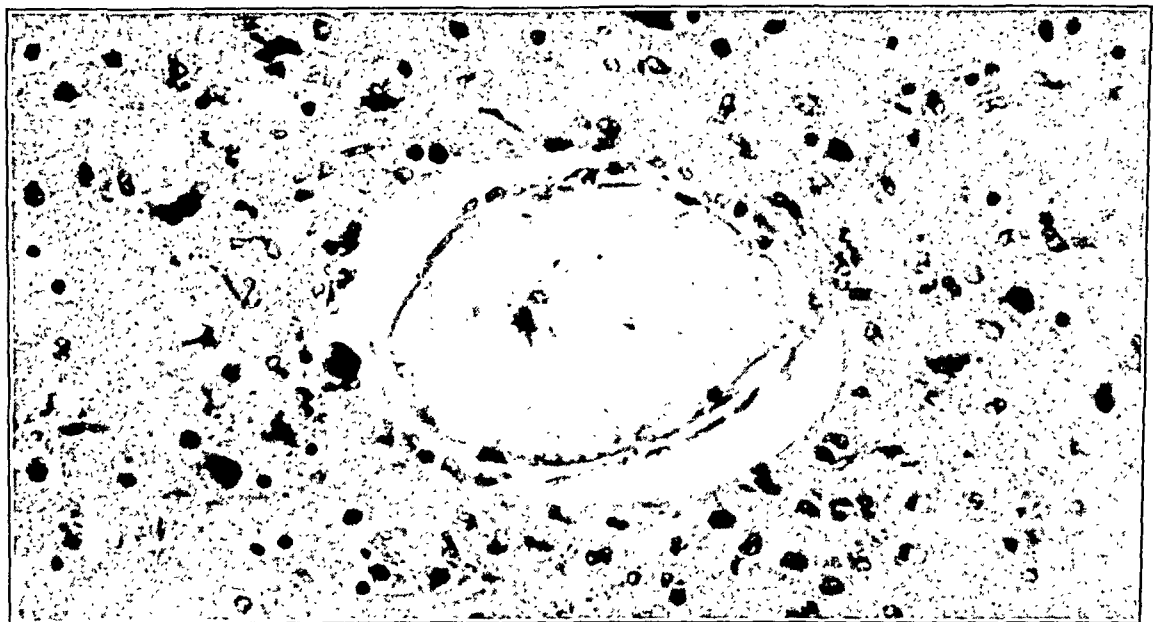
DESCRIPTION OF PLATES

PLATE 182

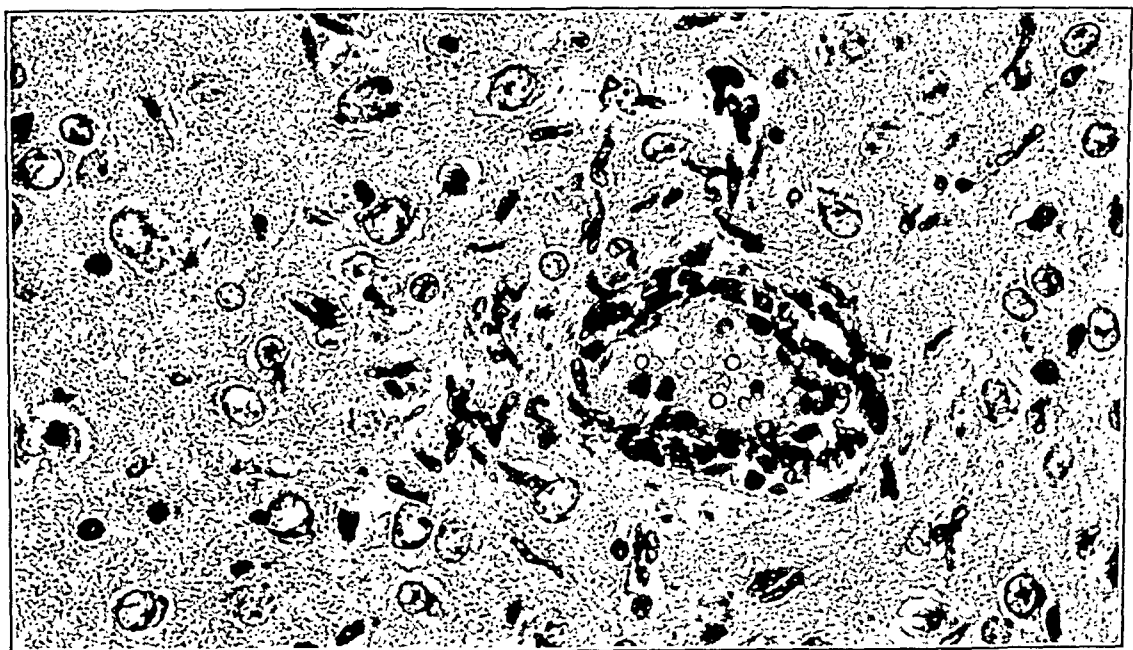
- FIG. 1. Vessel with collection of lymphocytes and mononuclear phagocytes in the perivascular space. The surrounding brain tissue is free from infiltration. Hematoxylin and eosin. $\times 700$.
- FIG. 2. Here the perivascular space is clear but in the brain tissue surrounding the vessel there is a collection of newly formed cells. At the left of the vessel there are several cells with curved and dumb-bell-shaped nuclei, which appear to be microglia. Several dark, shrunken nerve cells are apparent. Hematoxylin and eosin. $\times 700$.
- FIG. 3. A vessel with a perivascular collar of mononuclear cells. In addition, in the surrounding brain tissue there is a proliferation of microglia cells with irregularly shaped nuclei. Hematoxylin and eosin. $\times 700$.



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PLATE 183

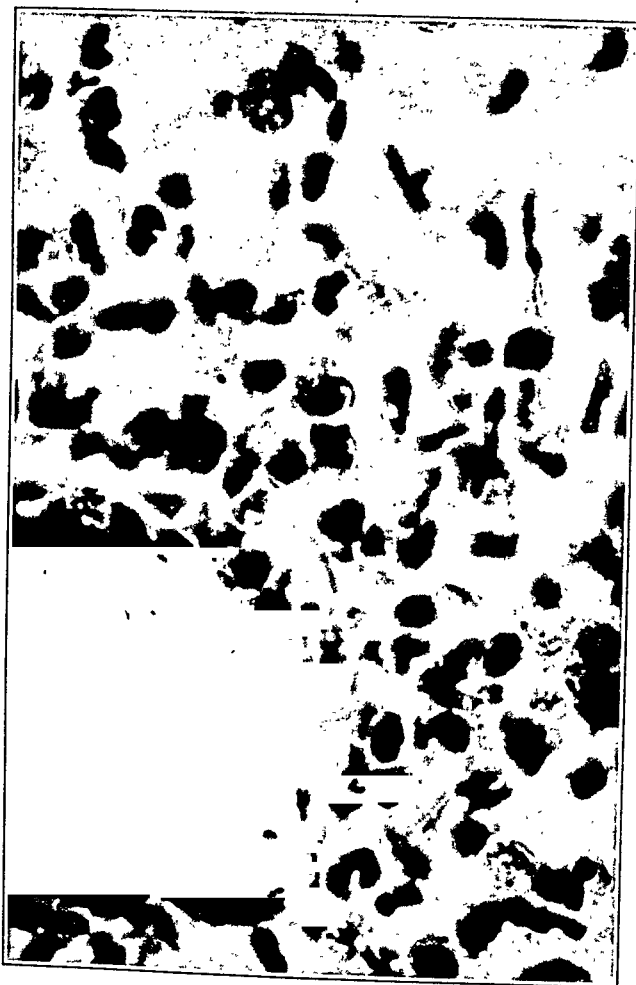
FIG. 4. A blood vessel is seen at the lower left, around which there is a collection of mononuclear and glia cells. Microglia are seen as irregularly shaped, elongated rods. In the upper right portion a microglia cell with processes is apparent. P. del Rio Hortega's silver carbonate method. $\times 700$.

FIG. 5. Degeneration of ganglion cells in Ammon's horn. In a circumscribed area the degenerated ganglion cells appear dark and homogeneously stained. Adjoining this area normal ganglion cells are seen. Hematoxylin and eosin. $\times 300$.

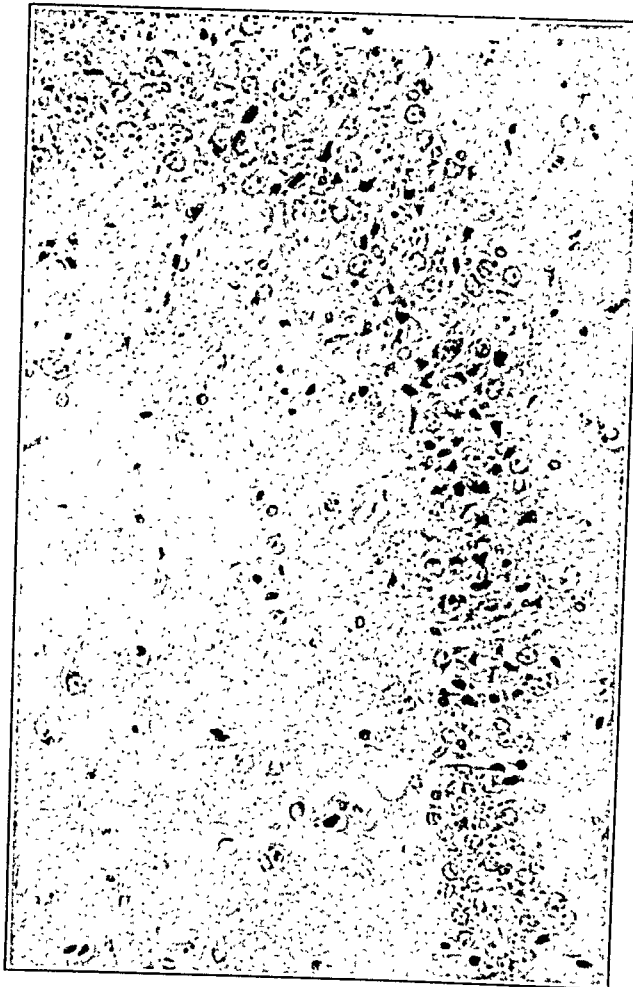
FIG. 6. Infiltration of meninges with lymphocytes and large mononuclear wandering cells. The infiltration extends down into the cortex around a vessel. Hematoxylin and eosin. $\times 300$.

FIG. 7. Small focal collection of mononuclear and glia cells in region of basal nuclei. Hematoxylin and eosin. $\times 300$.

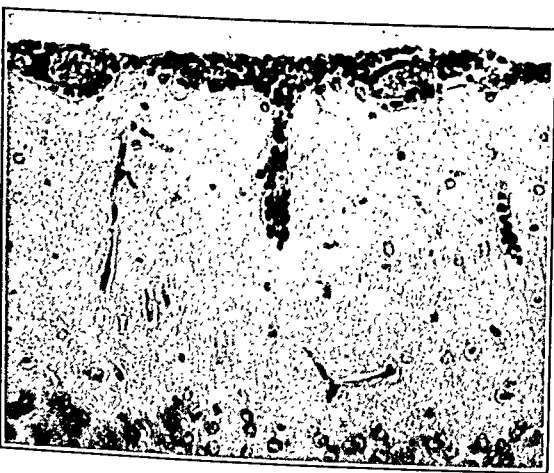
FIG. 8. Degeneration of motor cells in anterior horn of spinal cord. Proliferation of glia cells is also present. Hematoxylin and eosin. $\times 700$.



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* Abstract of paper presented at the meeting of the American Association of Pathologists and Bacteriologists held at Toronto, Ontario, March 29 and 30, 1934.

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